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DISEASES DUE TO PROTOZOAN PARASITES.

LEGER (M.) & VIENNE (M.). **Epizootie à trypanosomes chez les Bovidés de la Guyane française.** [Outbreak due to Trypanosomes among Cattle in French Guiana.]—*Bull. Soc. Path. Exot.* 1919. May. Vol. 12. No. 5. pp. 258–266.

Several forms of trypanosomiasis have already been described in South America in the form of more or less fatal epizootics or in some cases enzootics. "Mal de caderas" caused by *T. equinum*, discovered in 1901 by ELMASSIAN. has a very widespread distribution covering Paraguay, Bolivia, several provinces of the Argentine Republic and a large part of Brazil. The "peste boba" and "desrengadera" of Venezuela studied in 1905 by RAFAEL RANGEL are due to *T. venezuelense* MESNIL, 1910. The trypanosomiasis known as "murrina" occurred in Panama among mules recently imported from the United States and this was studied by DARLING and ascribed to a trypanosome, *T. hippicum*. Dourine, caused by *T. equiperdum*, has been identified in Chili by MOUFALLET and in Brazil in 1912 by SABOIA. All these trypanosomes have been hitherto encountered only in the equidae. No outbreak of disease due to flagellates has as yet been described in cattle in South America. In this paper the authors describe an outbreak of trypanosomiasis among oxen in a village in French Guiana. Before the occurrence of the outbreak this village contained about 180 head of cattle which were kept principally for the milk supply of the capital Cayenne, situated about ten kilometers away. These cattle were distributed in small groups. At the end of the epizootic only 85 survived, many of which were in an extremely poor condition. The disease probably first made its appearance towards the end of 1917 but its origin could not be discovered.

The symptoms exhibited by the affected animals were those of a progressive anaemia running a somewhat slow but almost invariably fatal course. Cattle were sometimes found infected with trypanosomes before they had shown any visible outward symptoms. The first symptom was loss of condition, which became gradually more marked up to the time of death although in some cases a temporary improvement was observed. Soon afterwards an oedematous swelling was

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observed affecting the dewlap and intermaxillary space. The swelling of these parts was generally very pronounced and might disappear for several days or weeks and then re-appear, and no relationship could be found between its presence and that of the trypanosomes in the peripheral blood. The appetite was maintained unimpaired almost up to the time of death. Anaemia was manifested by the pale appearance of the various mucous membranes. A heifer which had been affected for about six months showed only 4,800,000 red corpuscles per cubic millimetre instead of 8,000,000 to 9,000,000 as seen in normal cattle. The haemoglobin content fell to 55 per cent.; there was a quite appreciable leucocytosis (22,000 leucocytes per cubic millimetre). There was no distinct change in the morphology of the red corpuscles and the proportion of the leucocytes was as follows: neutrophile polynuclears 8 per cent., lymphocytes 64 per cent., large mononuclears 10·5 per cent., transitional mononuclears 16·5 per cent., eosinophiles 1 per cent. There was thus a marked mononucleosis, 91 per cent. (instead of 61 per cent. according to DENIER). In milch cows the milk yield was diminished by from two-thirds to three-quarters. There was frequent intermittent diarrhoea but no mucus or blood was passed in the faeces. Towards the end of the disease the animal appeared emaciated, had a rough coat, hair was lost over patches and the eyes and nostrils discharged. The gait became stiff, the animal performed circling movements and finally showed paresis of the hind quarters or was unable to get up, and died after a few days. Symptoms such as those seen in other trypanosomiases e.g., keratitis and ophthalmia, early paralysis of the hind quarters, lameness and respiratory troubles were not seen. No abortions occurred. It was difficult to estimate the total duration of the disease inasmuch as it was often not suspected until it had become pretty far advanced, but certain affected animals kept under observation were found to be still alive after six months. Other animals showed some improvement in condition during the rainy season when grass became more abundant.

The trypanosomes are found to be present in extremely variable numbers in the peripheral blood. They were found in large numbers both in animals still presenting appearances of good health and in others that showed marked anaemia and died a few days later. Usually in animals showing well-defined symptoms of the disease the organisms were rare or extremely rare and sometimes could not be discovered. It seemed that an intensive multiplication of the trypanosomes took place in the peripheral blood at the commencement of the disease and then they diminished quite appreciably in number and were sometimes extremely rare. Just before death the trypanosomes again multiplied considerably.

The trypanosomes are probably transmitted by means of Tabanidae, which infested the affected herds in great numbers. However, the authors failed to discover any flagellate forms in the intestines of some of these gad flies. The dimensions of the trypanosome were as follows: total length 22–23μ (including free flagellum, 6μ), breadth 1·5 to 1·75μ; from posterior extremity to centrosome 0·8μ, from centrosome to nucleus 5·5μ, from posterior to anterior extremities of the nucleus 3μ, from nucleus to anterior extremity 7·2μ, free flagellum 6μ. The body of the trypanosome was fusiform in shape

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with two pointed extremities, the posterior being a little less pointed than the anterior. The protoplasm, which was vacuolated and finely granular, stained of a bright blue colour. It was not loaded with chromophile granules. The nucleus was oval and almost central in position but a little nearer the posterior extremity. The centrosome, which was always very distinct, was rounded or oval and almost terminal; in many cases (1 out of 10) it occupied the very extremity of the parasite. A vacuole was observed almost constantly just anterior to the centrosome. The undulating membrane was not very wide or thrown into distinct folds. The free flagellum was visible only in very well stained preparations.

Forms undergoing longitudinal division were not rare. At the commencement of the process a lengthening of the centrosome was observed, which became rod-like (up to 1·5μ in length), and separated from the posterior extremity (sometimes 3μ apart).

The variations in size of the trypanosome were not extreme,—in length 16 to 26·5μ (including flagellum), in breadth 1 to 3μ. Other variations were as follows: from posterior extremity to centrosome 0 to 1·7μ, from centrosome to nucleus 2·8 to 6·5μ, nucleus 1·7 to 4·5μ, nucleus to anterior extremity 4·5 to 10μ.

An experimental study of this trypanosome is at present being undertaken by the authors, but unfortunately small laboratory animals are very difficult to obtain in Guiana. A heifer showing a not inconsiderable number of trypanosomes in its blood was kept under observation. After a few days the trypanosomes became very rare and after four months' observation the parasites were found to appear only intermittently in the blood and always in extremely small numbers (1 parasite in 6 or 8 smears). The animal, however, showed no signs of recovery. It lost condition, anaemia was very marked, and it also showed diarrhoea and commenced to lose its appetite. A guinea-pig inoculated subcutaneously with 2 c.c. of blood from the heifer did not become infected. The same guinea-pig received intraperitoneally three weeks later 1 c.c. of the same blood and subsequently showed no trypanosomes. Rousseau, L. (1918) similarly demonstrated the immunity of the guinea-pig towards the trypanosome of humpless cattle in the Cameroons [see this *Bulletin*, Vol. 6, No. 4, p. 264]. A dog inoculated subcutaneously with 2 c.c. of blood from the heifer did not become infected with trypanosomiasis.

The authors then compare the trypanosome with those occurring naturally in equines in South America. It is stated that no animal except the ox became infected during the course of the outbreak and, in particular, that the horse and the ass remained immune.

T. equinum of "mal de caderas" has a characteristic appearance; the centrosome is rudimentary and so small that it is difficult to detect; moreover, bovines of all mammals inoculated are those which show the least susceptibility towards experimental infection. No comparison need be made with dourine which constitutes a well-defined morbid entity. *T. hippicum* of mules at Ancon as studied by Darling and Laveran is broader than the bovine trypanosome, its undulating membrane is also better developed and although in both cases the centrosome is often quite terminal the posterior extremity is often blunt; finally among the numerous animals inoculated experimentally by Darling only the calf proved resistent.

T. venezuelense, the cause of the " peste boba " of horses in Venezuela is of approximately the same size as the cattle trypanosome ; the dimensions are stated to be 18 to 23μ by 1·7μ. The morphology of this flagellate has only been incompletely studied. MESNIL (1910) states that the parasite resembles that of surra but provisionally classifies it as a separate species. A fact of importance is that a large number of cattle are raised in Venezuela for slaughter, and that the epizootic described by RANGEL (1905) in various parts of the country attacks besides equines only dogs and certain wild animals.

A comparison is also made with *T. evansi*, which has never been identified in South America. The cattle trypanosome is evidently of the same type as that of surra and in order to differentiate the two parasites special laboratory tests, particularly cross immunity experiments, will have to be performed. However, there are a certain number of slight morphological differences between the two trypanosomes. The large forms of *T. evansi* attain 35μ in length, the Guiana trypanosome does not exceed 26·5μ. The nucleus of the surra trypanosome is somewhat nearer the anterior extremity whereas in the authors' trypanosome it was nearer the posterior extremity. The protoplasm of *T. evansi* is, moreover, said to have scattered in it large chromophile granules, which were not observed in the case of the other flagellate. The resistance of cattle towards surra, although not a matter of peremptory importance, is nevertheless not negligible. Recovery is the rule among cattle in India naturally infected (LINGARD) and in Mauritius the mortality from surra has not exceeded 25 to 30 per cent. among oxen, whilst it reached 100 per cent. in horses (DEIXONNE). No comparison need be made between the Guiana trypanosome and the large trypanosome *T. theileri* obtained by CARINI (1911) by making blood cultures from bovines in Brazil.

The authors conclude that the trypanosome described by them infecting naturally bovines, and only bovines, in French Guiana appears to be a new species and propose to denominate it *T. guyanense*.

VELU (H.). **Trypanosomiase des chevaux du Maroc. Guérison de la maladie expérimentale du chien par l'osarsan.** [Moroccan Horse Trypanosomiasis. Recovery of a Dog from the Experimental Disease after Treatment with Osarsan.]—*Bull. Soc. Path. Exot.* 1919. May. Vol. 12. No. 5. pp. 220–223. With 2 charts.

LAVERAN and others have published the results obtained in the treatment of trypanosome infections by means of the derivatives of diaminoarseno-benzine known as O_1 and OK_1 prepared by FOURNEAU and OECHSLIN of the Pasteur Institute, Paris. In his experiments upon the treatment of Moroccan horse trypanosomiasis VELU utilised the product known as osarsan manufactured by the Parisian commercial firm " Poulenc."

Some preliminary experiments performed in 1916 showed that a single injection (5 grammes in the horse, 1 gramme in the dog) was not sufficient to bring about recovery ; these doses caused the rapid disappearance of the trypanosomes from the circulating blood, but did not prevent the occurrence, sooner or later, of relapses. Treatment by means of a series of injections with increasing doses was then tried ;

a donkey affected with dourine recovered (1917) after having received three injections graded from 5 to 7 grammes over a month.

In 1918 four dogs infected with *T. marocanum* were submitted to treatment by intravenous injections of doses of from 9 to 10 centigrammes per kilo. body weight, repeated at intervals of from 7 to 9 days. From the results obtained the author concludes that osarsan administered after inoculation with trypanosomes and before the appearance of the organisms in the blood is capable of acting as a certain preventative. The dogs which had become infected and had shown trypanosomes in their blood were completely cured, and remained in good condition during an observation period of 9 months in three cases and 4 months in the remaining case. Since these preliminary experiments were performed other animals affected with Moroccan trypanosomiasis (dogs), with debab (dogs), and with dourine (horses) were treated with the same successful results.

Taute (M.) & Huber (F.). **Die Unterscheidung des *Trypanosoma rhodesiense* vom *Trypanosoma brucei*. Beobachtungen und Experimente aus dem Kriege in Ostafrika.** [The Differentiation of *T. rhodesiense* from *T. brucei*. Observations and Experiments from the War Zone in East Africa.]—*Arch. f. Schiffs-u. Trop.-Hyg.* 1919. June. Vol. 23. No. 11. pp. 211–226. With 2 maps.

This paper contains an account of some clinical observations and some remarkable experiments performed on man to ascertain the identity or otherwise of the nagana trypanosome of the domesticated animals with that causing sleeping sickness of man in Northern Rhodesia and Nyasaland.

This very important question has been much discussed by investigators on these diseases since the year 1913. Even before that date Ascenso (1911) attempted to transmit some unknown species of animal trypanosomes to man, and in 1912–1913 Taute endeavoured to infect himself with *T. brucei* in Portugese East Africa, with negative result, and concluded that this organism was not pathogenic for man. Kleine, Fischer and Eckard, and later Schilling and Schreck upheld this view. On the other hand Bruce and several other authors have maintained the view that sleeping sickness due to *T. rhodesiense* is simply an infection of man with *T. brucei*. There is indeed a remarkably close resemblance in almost every respect between *T. brucei* and *T. rhodesiense*. Both trypanosomes are naturally transmitted by the same flies, *Glossina morsitans* and nearly related species, both trypanosomes show the same morphological characteristics, the same pathogenicity for animals, the same virulence and drug fastness. The only distinction between them according to Taute lays in their markedly different behaviour towards man. Bruce, however, held that Taute's human experiments were not conclusive; he maintained that in the course of experiment, as in nature, *T. brucei* would not be pathogenic for every man but would behave differently towards different men. Bruce's view could be explained in two somewhat interdependent ways, viz., (1) *T. brucei* might be pathogenic for relatively few men, in whose bodies there would be no, or an insufficient quantity of, protective substances against the

organism, whilst on the other hand the great majority of human beings possess the required degree of immunity; (2) strains of *T. brucei* had gradually become accustomed to human serum and were now in the position to set up *T. rhodesiense*-sleeping sickness in man.

These hypotheses are contra-indicated by the natural incidence of the disease. Sleeping sickness in Rhodesia, Nyasaland and in the Rovuma area occurs in relatively small circumscribed foci whereas *T. brucei* is uniformly spread over very large extents of territory. The other conditions—climate, altitude, incidence of tsetse, race of inhabitant—present in the sleeping sickness centres no differences from those of the surrounding tsetse areas. It is also difficult to comprehend how in nature certain small localities often separated by some hundreds of kilometres from each other should contain some natives exhibiting a different degree of immunity from that of the remainder of the population of Central Africa. For, the immunity acquired in childhood by the bites of tsetse against *T. brucei* infection must be somewhat equally distributed throughout all the tsetse areas. KLEINE and his co-workers, in particular, found that in different heavily infected nagana districts an examination of 1,500 children showed no trypanosomes in any case.

If one adopts the view that the prime factor is not a difference in the immunity of the human population but that on the other hand differences in virulence may be exhibited by *T. brucei* and that it is capable of adapting itself to man, then one should find gradations in its infectivity towards human beings, for this accommodation could not possibly be brought about suddenly. Observations on the natural disease show that this is not the case in Nyasaland and Rhodesia. Human trypanosomiasis is found either as an acute disease or it is not found at all. A medium infection, comprising slight manifestations of the disease or apparently healthy carriers, is not to be found among human beings even in localities where the domesticated animals without exception become infected with nagana. The question as to why the adaptation of *T. brucei* towards man should only occur in sharply defined localities whilst the same parasite under exactly the same conditions otherwise should not be able similarly to adapt itself thus remains inexplicable.

During the military operations in German East Africa in which large masses of German-led troops were moved between Kilimandjaro and Quelimane, out of the thousands of troops and followers under von Lettow the first cases of trypanosomiasis in human beings only appeared during the period February to the beginning of April 1917, although since the beginning of the war no man could have escaped the bites of tsetse. On the other hand all the transport and slaughter animals belonging to the troops regularly became infected with nagana shortly after entering a tsetse area. The authors then proceed to give particulars with regard to the history and course of the disease in eight different foci of sleeping sickness infection identified by them in the territory laying north and south of the Rovuma River (the boundary line between " German " and Portuguese East Africa).

The experimental part of the authors' investigations appear to be very remarkable, inasmuch as they do not seem to have hesitated to employ large numbers of human beings for direct inoculation. Experiments in this connection upon the higher apes were commenced

by Taute in 1914 at Niansa on Lake Tanganyika but had to be interrupted owing to the war. It was found to be hardly possible to employ the large number of chimpanzees that would have to be utilised in order to obtain results of general value. According to BRUCE, experiments upon man could not be regarded as significant unless at least 100 persons were used whilst the possibility could not be excluded that different men might exhibit different degrees of susceptibility against *T. brucei*. A suitable opportunity for the employment of the requisite experimental material, it is stated, was afforded by the war conditions. The human beings first employed consisted of one of the authors (Taute) together with a number of coloured criminals, and later, after the first experiment had proved negative, both the authors and simultaneously a large number of native carriers were used. The coloured human beings were obtained from 11 different tribes and some of these came from localities—as, for example, in the case of the people from the Ssassawara district—in which *T. rhodesiense* was endemic. The authors employed not only healthy and fit men but also decrepit and sick people for their experiments. The sites for the experiments were chosen in places which did not exhibit any climatic conditions different from those of sleeping sickness areas. Two experiments (Nos. 5 and 6) were conducted within a focus of sleeping sickness near Chirumba.

As the source of *T. brucei* for the human infections, the authors used four naturally infected horses and two mules. Laboratory strains were thus not employed. "Posterior nuclear forms" were found among the trypanosomes in three of the naturally infected horses. As control animals, to be tested with regard to their susceptibility towards the strains of *T. brucei* employed, the authors utilised trypanosome-free rats, dogs, and one goat. Some of these control animals were unfortunately lost at an early date owing to the very difficult conditions of working but fortunately this had no important bearing upon the results. The appearance of "posterior nuclear forms" upon sub-inoculation of *T. rhodesiense* and *brucei* into experimental animals in these and other experiments were, in concordance with the recent results of HUGHES, of somewhat regular occurrence but still not absolutely constant. This confirms KLEINE and Taute's earlier view that "posterior nuclear forms" are brought about only subsequent to a rapid multiplication of the parasites. The description of the first of these experiments is somewhat as follows:—

(Expt. 1).—Site of Experiment: Camp near Mwiti, German East Africa, about 400 metres above sea level in the Rovuma River district.

Time of Experiment: End of October and beginning November, 1917.

Source of *T. brucei* Strain: Naturally infected mule K suffering from nagana for about a year. Treated with 4 atoxyl and 5 tartar emetic injections, for the last time three months previously. The mule which had made a good recovery from the original infection had recently again become poor in condition. Dimorphic trypanosomes of the type *T. brucei* were regularly found in the blood, which had proved to be pathogenic upon subsequent inoculation into rats and dogs.

Persons experimented upon: No. 1: Dr. T.

No. 2–10: Nine native criminals from the sleeping sickness area in the Upper Rovuma District near Ssassawara.

Five of these human beings were in good general condition; the others were in rather a low state on account of chronic malaria, ankylostomiasis

and chronic dysentery. After frequently repeated blood examination no trypanosomes were found on any occasion in these people.

31.x.17. In the blood of the mule K numerous trypanosomes of the dimorphic type. The blood of the experimental men nos. 1–10 free from trypanosomes as hitherto.

Experimental persons nos. 1–10 were each inoculated under the skin of the chest with 5 c.c. of blood from mule K immediately after the blood had been obtained.

Simultaneously three trypanosome-free rats were each injected with 0·5 c.c. of blood from the mule K under the root of the tail.

2.xi.17. Mule K given two grammes atoxyl subcutaneously.

3.xi.17. Mule K given 1 gramme tartar emetic intravenously. In the blood of the mule K well developed trypanosomes in somewhat increased numbers were found (the trypanosome strain thus shows a considerable degree of resistance against arsenic and antimony).

10.xi.17. Out of the three rats inoculated with blood from mule K on the 31.x. the two still alive were found to be affected with trypanosomes of the dimorphic type. Posterior nuclear forms could not be found on the 10.xi. or on the following days.

1.xi. to 14.xi. The experimental persons nos. 1–10 after frequently repeated blood examination proved to be entirely negative, and showed no clinical signs of infection. The observation on persons nos. 2–10 had to be discontinued on the 14.xi. for reasons beyond the authors' control; experimental person no. 1 after blood examinations extending up to January 1919 proved to be continuously free from trypanosomes.

The details of five similar experiments subsequently performed are then given, the results obtained as far as infection of human beings was concerned being constantly negative though the period of observation was in each instance eight weeks.

These six experiments thus gave results in full concordance with those of Taute's early experiments in that different strains of *T. brucei* obtained from quite different localities (Lubimbinu, Mwiti, Chiwata, Chirumba) after inoculation into two Europeans and 129 negroes in all did not bring about a single case of human infection. The inoculated persons subsequently neither showed any symptoms nor did they become carriers of the parasite. The view upheld by KLEINE and his school with regard to the important *T. brucei—rhodesiense* question is thus regarded as the right one.

The difference thus proved to exist between the two trypanosomes must find its application in combating sleeping sickness.

It had hitherto been maintained that the existence and spread of nagana in animals had a bearing upon sleeping sickness in man, but in view of the results obtained one is now justified in applying the same comparatively simple means for eradicating *T. rhodesiense* as suggested by KOCH (R.), KLEINE (F. K.), BRUCE (D.), and their co-workers in the case of *T. gambiense*. In the first place it is necessary to map out carefully the various centres of infection and then to adopt quarantine methods, including isolation of infected persons or prevention of movement into or out of the affected centres. Treatment of the infection is difficult owing to the high degree of drug-fastness of *T. rhodesiense* in a large percentage of cases.

Of the highest importance in eradicating *T. rhodesiense* is the much discussed question as to what part game and domesticated animals play as carriers of infection. It has been demonstrated that the organism is pathogenic towards all the domesticated animals. According to KINGHORN and YORKE up to 16 per cent. of the game of the Luangwa valley, North-Eastern Rhodesia, is affected with *T. rhodesiense*.

This statement quite lacks foundation and is apt to lead to false or to quite exaggerated ideas, although it is possible that the game in certain circumstances may act as a carrier of *T. rhodesiense* infection. The destruction of all game in all tsetse areas, however, is held to be both unnecessary and impracticable. The authors quote with approval the British Inter-Departmental Committee which issued recommendations for the limited extermination of game. The ideal to be obtained would naturally be the extermination of *Glossina morsitans*. This would be impossible to achieve throughout the tsetse areas but it is highly necessary and desirable that it should be performed in sleeping sickness areas. With the development and cultivation of larger tracts, however, the fly will become gradually pushed back.

HORNBY (H. E.). **A Few Notes on Autoagglutination.**—*Vet. Jl.* 1919. June. Vol. 75. No. 6. pp. 207–211.

If a drop of blood from a normal horse is spread as a thin film between a slide and a cover glass the red corpuscles are seen on microscopic examination to tend to form rouleaux. These chains intertwine and anastomose readily to form various sized masses of red cells in most of which, however, rouleaux formation is not lost. Between these masses a few isolated rouleaux and free cells may be seen. Many observers have noted that the picture presented by a film from a horse or other animal affected with trypanosomiasis is sometimes different. The corpuscles then present the phenomenon known as autoagglutination, a number of islets of varying size and shape being formed while rouleaux structure is completely lost and even the cellular nature of the masses is apparently obscured. This phenomenon was first detected in 1898 by KANTHACK, DURHAM, and BLANDFORD whilst working with experimental nagana. CHRISTY (1904) and MARTIN and LEBOEUF (1908) noticed it in cases of human trypanosomiasis, TODD (1910), however, found that in many cases of sleeping sickness no autoagglutination could be observed; BECK (1911) found that it might be observed on one day and not on another in patients suffering from this disease; WERNER (1913) described an acute case which ran its course without autoagglutination.

In animals similar fluctuations have been observed. LAVERAN holds that the phenomenon depends less upon the species of trypanosome than upon the species of host; thus, according to this author, the horse, dog, and cat may show strong autoagglutination. The phenomenon may occur in infections not due to trypanosomes and it appears that trypanosome-free apparently healthy men show slight or, in rare instances, strong degrees of autoagglutination. In healthy animals, however, the phenomenon was seldom seen by YORKE. The various degrees of intensity of the phenomenon were thus indicated by MARTIN and LEBOEUF and later by DUBOIS:—

(1) Strong. Coarse clumping into shapeless islands or packets in which rouleaux formation is not visible.

(2) Distinct. Large clumps, but with contours of rouleaux still detectable here and there.

(3) Weak. The masses show obvious rouleaux formation. No sharp line can be drawn, it is stated, between this grade and the normal appearance of blood.

The phenomenon was further studied by YORKE in 1911, NATTAN-LARRIER having previously ascertained that the addition of sodium chloride solution rendered it more pronounced. YORKE found that the temperature was the most important factor in its production, the phenomenon being absent at 37°C, well-marked at 18-21°C, and at its optimum at 0°C. Autoagglutination, it was stated, was a property of the plasma and depended upon the enrichment of the normally occurring auto-, iso-, and heteroagglutinins. The agglutinin could be extracted from the massed erythrocytes by the addition of 0·9 per cent. sodium chloride solution. By rapid centrifugation with citrated saline or by shaking with glass beads at 37°C one could obtain a plasma so rich that on ice it would agglutinate even when diluted 15 to 20 times. Normal plasma would not cause agglutination if diluted with more than double its volume.

MARTY (1917), working with human blood, again dealt with this subject [see this *Bulletin*, 1917, Vol. 5, No. 3, p. 160] and he attached more importance to the structure of the haemolute at the periphery of the preparation than to the clumps at the centre. The size of the drop to be studied was stated to be of great importance. It should cover one half to three quarters of the area of the cover glass and not run to the edge. A trace of 1–1,000 potassium carbonate solution was stated to disintegrate the pseudo-agglutinated clumps that sometimes occur in other diseases but to fail to disintegrate clumps truly agglutinated as the result of trypanosomiasis.

Hornby then quotes some interesting observations by MINES upon the electrolytic properties of the blood corpuscles of the dog-fish in various concentrations of cerium chloride. The observations of YORKE and of MARTY, it is stated, leave but little doubt that the phenomenon of autoagglutination occurs likewise as the result of absorption of electrolytes.

The author's own work consisted of a series of observations to ascertain whether autoagglutination was pathognomonic of trypanosomiasis and whether it was of any value as an aid to diagnosis. Bovine blood was found never to show either distinct rouleaux formation or autoagglutination. Only a few observations were made on the small ruminants and felines. The author's attention was early directed to the phenomenon as it occurred in equines affected with trypanosomiasis. To determine whether it was a constant feature in such circumstances the author made cover-glass preparations from 29 fly-struck mules. Of these the blood of five showed autoagglutination and trypanosomes, seven showed autoagglutination without trypanosomes, seven showed no autoagglutination though trypanosomes were present, ten neither autoagglutination nor trypanosomes.

In another experiment the blood of 14 fly-struck equines (horses, mules, and donkeys) gave the following results:—Three showed both autoagglutination and trypanosomes, two showed autoagglutination without trypanosomes, three showed no autoagglutination though trypanosomes were present, six showed neither autoagglutination nor trypanosomes.

A number of daily observations were next made on the blood of horses, mules, and donkeys affected with either *T. brucei* or *T. congolense*. It was found that neither the species of trypanosome nor the

species of equine had any bearing upon the phenomenon, which might be present on one day and absent on the next; there was no co-relation between the presence or absence of the parasite and the existence of autoagglutination. Likewise there was no co-relation between the clinical symptoms and the presence or absence of the phenomenon. In fly-struck equines the phenomenon would thus be exhibited in about half the cases examined and the chances do not appear to depend upon whether the animal is suffering from an acute, sub-acute, or chronic attack, whether the body temperature is high or low, or whether the parasite is *T. brucei* or *T. congolense*.

The author endeavoured to ascertain whether the presence of the phenomenon was a sure sign of an animal being fly-struck. The blood of 20 healthy horses in good condition was examined and of these eight showed weak autoagglutination and in the remaining 12 the corpuscles in some cases showed a tendency towards autoagglutination so that the phenomenon could not definitely be asserted to be absent. In the case of 18 healthy mules the blood in six showed weak autoagglutination, in five the phenomenon was absent, in the remaining seven the results were intermediate. From these findings it was concluded that "strong" or "distinct" autoagglutination was rare in healthy equines.

The blood of more than 100 equines including two affected with filariasis was subsequently examined. In three only was marked autoagglutination observable, without any signs of trypanosomiasis. One of these was a donkey affected with coronitis, which caused shedding of the hoof; the second was a horse with a wound of the fetlock attributed to snake bite; the third was an apparently healthy horse.

The author emphasizes the importance of MARTY's precautions in making the film of a suitable size, taking absolutely fresh blood, and using clean glass. The occurrence of the phenomenon to a marked degree, it is concluded, suggests the possibility, or even the probability, of trypanosome infection.

HORNBY (H. E.). **The Diagnosis of African Equine Trypanosomiasis.** —*Vet. Jl.* 1919. June. Vol. 75. No. 6. pp. 218–225.

The difficulty of diagnosing with certainty this class of disease, it is stated, was a cause of useless expenditure of public money during the East African campaign. Large numbers of fly-struck and therefore doomed animals were sent long distances by road, rail, and sea to Base Veterinary Hospitals where sometimes they were treated for many weeks before their condition became such as to render the nature of their disease sufficiently obvious to justify slaughter. If a diagnosis had been made at the Advanced Veterinary Hospitals the animals could have been destroyed there and subsequent expense saved.

In a previous communication [see preceding number of this *Bulletin*, p. 53], the author pointed out the futility of attempting curative treatment with the means at present at our disposal. The most economical method of dealing with affected equines is to work them as long as this can be done without cruelty and then to destroy them.

Excluding the cause of surra, which disease is not essentially an African one, three trypanosomes are responsible for equine disease in Africa, viz., *T. brucei*, *T. congolense*, and *T. vivax*. All three trypanosomes set up fatal diseases of the same nature differing only in the fact that the course of the one set up by *T. brucei* is generally rapid whereas that set up by either of the other two is more frequently chronic. Some mules, however, sometimes show more resistance to *T. brucei* than horses do to the other trypanosomes. Acute cases can generally be rapidly diagnosed by demonstration of the parasite. It is the chronic cases which present difficulty. A cursory examination, which alone can be performed in dealing with large numbers at one time, is not sufficient for the basis of a diagnosis involving destruction.

Lanfranchi (1915) [see this *Bulletin*, 1915, Vol. 3, No. 2, p. 41] stated that a specific local reaction could be obtained by the instillation or injection of an extract of the infecting trypanosomes. The author attempted to manufacture such an extract and injected it into the eyelid of the donkey from which the parasites were obtained. The result was negative.

One thus has to rely upon clinical and microscopic examination and the author's procedure in the diagnosis of suspicious cases is thus, first, to obtain as full a history as possible of the animal enquiring particularly as to whether it has passed through a fly-belt and at what time, and, secondly, whether the animal is losing condition in spite of a good and satisfied appetite. The second question is regarded as one of great importance inasmuch as there are few conditions other than trypanosomiasis in which an animal eats well and yet steadily loses condition. The animal is then kept under the best hygienic conditions available for two or three days in order to allow it to recover from any slight indisposition due to fatigue or mismanagement from which it might be suffering at the time of admission. The existence of muscular or nervous weakness, the appearance of the membrana nictitans, the presence or absence of lymphadenitis, keratitis, or other lesion associated with the suspected disease is then noted. Darling (1912), dealing with the equine disease due to *T. hippicum*, described it as an intoxication resulting in cellular degeneration and necrosis, injury to or destruction of the continuity of the endothelium with consequent effusion, oedema and terminal ecchymoses. This description applies also to African trypanosomiasis and it is on the findings of symptoms suggestive of such a state that in the absence of the parasite the author bases his diagnosis. If after completing his examination he finds that he has as a subject an animal known to have been through a fly-belt, in poor condition with, perhaps, a history of wasting, if, in addition, it has pale yellow or blotched mucous membranes and has tachycardia, and if in spite of these symptoms of illness it eats well, is in no pain, and excretes normally, then he feels justified in saying that the subject is a "fly-suspect."

The next step in diagnosis is to examine the blood, a small drop of which is obtained by incising the tip of the ear and placed between a clean slide and cover glass so that the drop covers an area of half to three quarters that of the cover glass. The preparation is examined with a $\frac{1}{6}$ inch objective and if one trypanosome is discovered the task of diagnosis is completed. Out of 29 mules examined affected with the

sub-acute and chronic condition the first day's search gave 12 positive results, the next day's five more, and the third day's one more, that is, 11 were negative on three successive days. A number of records of typical individual cases are appended showing that the parasites are generally demonstrable in acute and sub-acute cases whereas they may not be evident in chronic cases for many weeks at a time. If the parasites are not discoverable a note is made of the presence or absence of marked autoagglutination, the presence of this phenomenon being somewhat indicative of trypanosomiasis.

Examination of gland juice presents no advantages over the examination of fresh blood and it is frequently difficult to obtain. Except for the recognition of the species of trypanosome, stained films are inferior to fresh ones for the purposes of diagnosis inasmuch as the stained cells give no indication as to the nature of the disease and the volume of blood examined is less, hence the chances of discovering a rare trypanosome are correspondingly smaller. In cases where the parasites are not demonstrable the animal's temperature is taken on three or preferably four successive days. The fever in chronic trypanosomiasis when the parasites are not demonstrable is of a low intermittent or remittent nature. Afebrile periods occur in such cases but very rarely does a fly-struck animal go for more than three days without showing an evening temperature of 102° F. or over. At the end of three or four days the temperature chart is examined and the animal and its blood re-examined. If the blood is still negative then the diagnosis must be made on the strength of the history and clinical symptoms. This procedure would enable an experienced man to arrive at a sufficiently accurate diagnosis although it might be wrong in a small percentage of cases.

[The author does not deal with experimental animal inoculation as a means of diagnosis. He probably considers this as being impracticable in the conditions in which he worked.—Ed.]

Nöller (W.). **Beitrag zur Kenntnis des Schaftrypanosomas. Vorläufige Mitteilung.** [A Study of the Sheep Trypanosome. Preliminary Note.]—*Arch. f. Schiffs.-u. Trop.-Hyg.* 1919. Mar. Vol. 23. No. 5. pp. 99–100.

The sheep trypanosome has hitherto been found by only two observers. Woodcock in 1912 discovered a living trypanosome in sheep blood and Behn found in 1911 and 1912 some trypanosomes in thick cover glass preparations taken from some sheep in Thuringia. The cultivation of the parasites from sheep blood has not yet been accomplished.

It has been found that in Germany nearly all the so-called "sheep keds" (*Melophagus ovinus* L.) are infected with the trypaniform flagellates seen by L. Pfeiffer in 1895 and named *Crithidia melophagia* by Flu in 1908. These flagellates are so remarkably similar to the developmental stages of the trypanosomes obtained from biting flies resembling the sheep ked that some authors (Woodcock, Behn) have held that they represent only developmental forms of the sheep trypanosome which may be encountered as sparingly in the blood of the sheep as *T. theileri* is found in the blood of cattle. This opinion appears to be supported by the experiments of Chatton and Delanoë, and of Cauchemaz.

The connection between the two parasites may be demonstrated in three ways :—

(1) By artificial infection of trypanosome-free lambs with keds containing the flagellates ; other blood-sucking insects must naturally be excluded in this test. The careful carrying-out of this experiment would involve considerable expense and the construction of an absolutely insect-proof cage.

(2) By the examination of the blood of lambs during the early lambing season in flocks infected with and free from keds. A fresh infection could be found in this way. Tabanids and stomoxyds would be excluded by examining during the coldest part of the year.

(3) By the cultivation of the sheep trypanosome and comparison with the cultures of the ked crithidia, which were grown on plates by the author in 1917 [see this *Bulletin*, 1917, Vol. 5, No. 3, p. 168].

A systematic examination of the blood of winter lambs from some flocks in Thuringia has been performed by the author and the results are promised in a further publication.

Experiments performed in connection with the third heading mentioned above gave rise to a rich culture from the blood of a sheep infected with keds, the media used being blood-broth tubes (equal parts of nutrient broth and defibrinated sheep blood) and sheep-blood agar tubes maintained for eight days at a temperature of 30° C., a temperature which was shown to be the optimum for the growth of the ked crithidia (Nöller 1917). Comparison with the ked crithidia showed that both organisms exhibited the same morphological characters and could be differentiated from the cattle trypanosome. A striking characteristic of the cultural forms of the sheep trypanosome and the ked crithidia was the marked development of the undulating membrane, which was broad and only carried a short free flagellum.

The sheep trypanosome has thus to be considered as a distinct species transmitted by the sheep ked, and manifesting itself as "*Crithidia melophagium*" Flu, 1908. This trypanosome is obtainable from the Institut für Schiffs- und Tropenkrankheiten, Hamburg. Further transmission experiments are being made on flocks in central Germany in order to settle beyond doubt the life history of the trypanosome. A detailed publication is promised in another journal.

DUBIN (Harry). **Studies of Urobilin Elimination in the Normal and Anemic Dog.**—*Jl. Exper. Med.* 1918. Sept. Vol. 28. No. 3. pp. 313–318. With 4 tables.

In congenital haemolytic jaundice and pernicious anaemia splenectomy is followed by a decrease in the elimination of urobilin in the faeces. As this decrease is coincident with an improvement in the blood picture it is generally assumed that the change in urobilin degeneration is due to decreased destruction of blood. The experiments described by the author in this paper were undertaken in order to obtain definite evidence upon this point. *Trypanosoma equiperdum* was employed to produce in dogs a continuous blood destruction, which, as it endures over long periods of time, may be considered as typical of a chronic experimental anaemia. The elimination of urobilin was studied in the normal and splenectomised animals and in the anaemic

animal both before and after splenectomy and treatment by arsenobenzol. The author summarises his results as follows :—

"1. The output of urobilin is increased in experimental trypanosome anemia presumably as a result of the increased blood destruction.

"2. The administration of salvarsan (arsenobenzol) during the anemic period, if it checks the blood destruction, reduces the urobilin elimination, but this result does not follow unless the blood picture improves.

"3. Following splenectomy in the normal dog an increase in the urobilin elimination of varying degree occurs, the significance of which is doubtful.

"4. Splenectomy during the period of anemia does not cause a decrease in urobilin elimination.

"The experiments support the view that the elimination of urobilin may be considered as an index of blood destruction, but they do not explain the decrease in the elimination occurring in man in certain forms of hemolytic anemia following splenectomy."

SERGENT (Edm. & Et.) & LHÉRITIER (A.). **Passage de trypanosomes de la mère au foetus dans le "Debab."** [Passage of Trypanosomes from Mother to Foetus in *Debab.*]—*Bull. Soc. Path. Exot.* 1919. Apr. Vol. 12. No. 4. pp. 177–178.

In this short note the authors give an account of two experiments upon the transmissibility of the *debab* trypanosome—*T. berberum*—across the placental filter in the pregnant camel. In the first experiment a camel inoculated with the trypanosomes commenced to show fever on the third day, trypanosomes appeared in its blood on the fifth day, and it aborted 36 days after the inoculation. The blood of the young camel, which was born dead did not show any trypanosomes on microscopic examination, but the blood was capable of infecting a dog by intraperitoneal inoculation.

In the second experiment the inoculated camel showed fever on third day, trypanosomes on the fifth day, and aborted on the 61th day after inoculation. The blood of the young camel which was born dead, showed no tryanosomes upon microscopic examination but infected two dogs by intraperitoneal inoculation. *T. berberum* is thus capable, it is stated, of passing through the placental filter.

CHALMERS (Albert J.). **The Classification of Trypanosomes.**—*Jl. Trop. Med. & Hyg.* 1918. Nov. 15. Vol. 21. No. 22. pp. 221–224.

"Even the casual reader cannot fail to be impressed with the enormous number of species of very diverse form and different life-history which are included in the genus *Trypanosoma* Gruby 1843." The term *Trypanosoma*, according to the author, should be restricted to the original meaning, viz. ; to a trypaniform parasite of relatively large size and slow movement, generally found in the blood of cold-blooded vertebrates and spread by leeches. We should then have a clearly defined genus *Trypanosoma sensû stricto*, and a very large number of species comprised in an ill-defined genus *Trypanosoma sensû lato*.

The new classification proposed is as follows :—

A. Live *only* in a definitive invertebrate host :—
Tribe 1, *Cystotrypaneae*, new name.
Type genus : *Cystotrypanosoma* Roubaud, 1911.
Type species : *C. intestinale* Roubaud, 1911.

B. Live in a definitive invertebrate host and in a cold-blooded intermediate vertebrate host :—

Tribe 2. *Trypanosomeae*, new name.
Type genus : *Trypanosoma* Gruby, 1843.
Type species : *T. rotatorium* (Mayer, 1843).

C. Live in a definitive invertebrate host and in a warm-blooded intermediate vertebrate host :—

Tribe 3. *Trypocastellanelleae*, new name.
Type genus : *Castellanella*, new name.
Type species : *C. gambiensis* (Dutton, 1902).

The three tribes are defined and examples given.

The pathogenic mammalian trypanosomes—which belong to the tribe *Trypocastellanelleae*—the author divides into two genera, as follows :—

I. Genus *Castellanella*, new name.
Definition.—Trypocastellanelleae with the definitive host a fly and the intermediate host a warm-blooded vertebrate. Without reproduction by schizogony in the vertebrate host, in which it is polymorphic, with granular cytoplasm, small kinetonucleus, and well-developed undulating membrane. Final stage in the definitive host is in the salivary glands. Infection (from fly to man) is inoculative and transmission (from man to fly) is ingestive.
Type species.—*Castellanella gambiensis* (Dutton, 1902).
Other species.—The other species of the genus may be differentiated as follows :—

A. *Posteriorly nucleate in rats* :—
I. Will not infect man. Kills an animal immunised against *C. rhodesiensis*—*C. brucei.*
II. Infects man. Kills an animal immunised against *C. brucei.*—*C. rhodesiensis.*

B. *Not posteriorly nucleate in rats* :—
I. Infection only known to proceed direct from intermediate vertebrate host to intermediate vertebrate host during coitus—*C. equiperdum.*
II. Infection by means of a definitive host :—
(*a*) Definitive host not a tsetse fly—*C. evansi.*
(*b*) Definitive host a tsetse fly :—
(1) Causes chronic and often mild infection in man—*C. gambiensis.*
(2) Causes acute and severe infections in man—*C. castellanii.*

II. Genus *Duttonella.* New name.
Definition.—Trypocastellanelleae with definitive host in a fly and intermediate host in a warm-blooded vertebrate. Without reproduction by schizogony in the vertebrate host, in which it is monomorphic. With non-granular cytoplasm, a large kinetonucleus, which may be terminal or sub-terminal, with or without a well-developed undulating membrane. Movements active. Final stage in the definitive host is confined to the proboscis and hypopharynx.
(*a*) Kinetonucleus large and terminal. Undulating membrane poorly developed and simple. Invertebrate host a glossina :—
(1) Rats refractory—*Uniformis.*
(2) Rats suspectible—*Vivax.*
(3) Only equidae and ruminants susceptible—*Caprae.*
(*b*) Kinetonucleus prominent and subterminal. Undulating membrane well developed :—
(1) Small, 8–18 microns ; found in cattle—*Pecorum.*
(2) Larger, 14–24 microns ; found in monkeys—*Simiae.*

The last portion of the paper concerns the classification of the various pathogenic trypanosomes of man.

SCHWETZ (J.). **L'identité des conditions géo-botaniques des gîtes à pupes de la *Gl. palpalis*, de la *Gl. fusca*, de la *Gl. brevipalpis*, de la *Gl. pallidipes* et de la *Gl. morsitans*.** [Identity of the Characters, Geological and Botanical, of the Pupal Shelters of *Glossina palpalis, fusca, brevipalpis, pallidipes*, and *morsitans*.]—*Bull. Soc. Path. Exot.* 1919. May 14. Vol. 12. No. 5. pp. 234–238.

The systematic study of the haunts of tsetse-flies is of relatively recent date. Two shelters containing numerous pupae of *G. palpalis* were discovered and described by RODHAIN and BEQUAERT in Katanga. Numerous shelters of the pupae of *G. morsitans* were found in Nyasaland and described by LAMBORN; but, as far as can be ascertained, little is as yet known concerning the sites for which *G. brevipalpis, pallidipes*, and *fusca* exhibit a predilection for the deposition of their larvae. In the course of a minute study upon the habits of these five species of Glossina undertaken during the three years 1916–1918 the author was able to discover numerous pupae of all these flies. This work was undertaken in the northern portion of the Province of Katanga, Belgian Congo, between the River Zualaba-Congo and its large tributary the Zomami, i.e., in the region of lat. 6° S. and between long. 25° and 27° E. A locality was chosen in which the five species of flies in question were found simultaneously in considerable number. The pupae of *G. palpalis* were most readily discoverable whilst on the other hand those of *G. fusca* presented most difficulty. Shelters occupied by the pupae of two or even three of the species were often found.

The characters found to be absolutely essential in a pupal shelter for all species were (1) a dry light mellow soil, and (2) shade. The shelters were also found to present some secondary special characters depending upon the fact that each of the various species of Glossina lived in a locality with a particular kind of vegetation.

G. palpalis is only to be found along the wooded banks of watercourses and thus the pupal shelters are to be found in suitable localities on the river banks. This fly prefers sandy localities for the deposition of its larvae but in places where pure sand is not to be found it is content with sand mixed with mould, or even with mould alone on condition that it is loose; inasmuch as the immediate borders of the watercourses are often flooded during the rainy season the fly only deposits its larvae in such places during the dry season, and thus at the beginning of the dry season living pupae are to be found almost exclusively whereas at the end of the same season dried pupae are usually only to be found in these places.

In cases where wooded banks of streams are inhabited by *G. brevipalpis* or *fusca* their pupae are found in places similar to those of *G. palpalis*.

G. morsitans, whilst it does not avoid the vicinity of water where the forest growth around it is not too thick, inhabits usually vast moderately wooded, even waterless, stretches. The same is the case with *G. pallidipes*. *G. brevipalpis* inhabits the densely wooded banks of rivers and is capable of adapting itself also to the localities frequented by *G. morsitans* and *pallidipes*. *G. fusca*, which only inhabits large tracts of forests, may also be found in the vicinity of water.

The search for the pupae of these flies was attended with a certain number of difficulties but they were found to exhibit a marked

predilection for large fallen or uprooted tree trunks lying horizontally or obliquely to the surface of the ground or raised a few centimetres away from it. All the conditions required were to be found underneath or on the sides of these fallen trees inasmuch as they afforded protection from sun and rain and the soil at such places was not damp or hardened. Underneath one of these trunks the author found as many as 200 pupae of *G. fusca*.

A thin undergrowth was found to be the most suitable; a dense undergrowth tends to make the soil too compact whereas in the absence of undergrowth no support is given to the flies at the time when the larvae are expelled.

Although the conditions enumerated above are those ideally suitable isolated or scattered pupae may be found in numerous other situations.

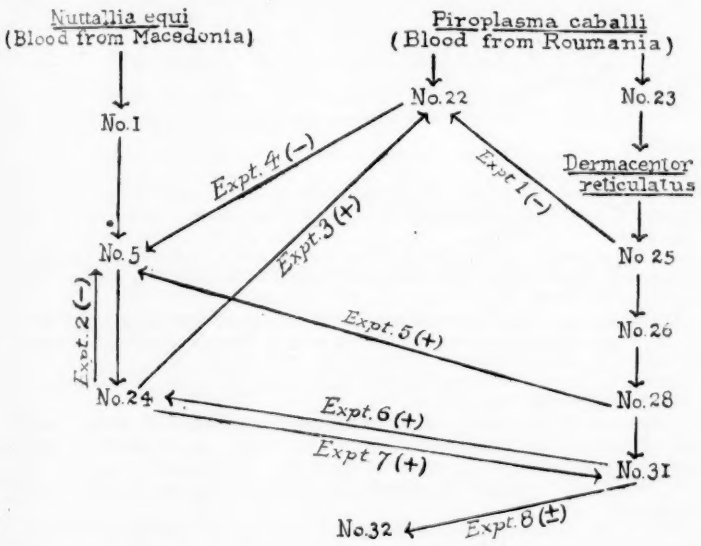
DUKE (H. Lyndhurst). **Some Observations on the Bionomics of *Glossina palpalis* on the Islands of Victoria Nyanza.**—*Bull. Entom. Res.* 1919. Mar. Vol. 9. Part 3. pp. 263–270.

On arrival at Nsazi Island on Victoria Nyanza in July 1918 to carry out some experiments with *G. palpalis* and rinderpest the author was at once struck with a marked diminution of the numbers of the fly on these and the neighbouring islands, a fact that was also noticed by the followers of FISKE and CARPENTER, who worked on these Islands from 1911 to 1916, when they were noted for the great numbers of tsetse. The diminution appeared to be due to the recent rise in the level of the Lake, which attained its maximum in June 1917, and in some places completely covered the great majority of the sandy beaches especially favoured by the fly for the deposition of larvae as noted by FISKE in the Royal Society Commission Report. Duke strongly advises the systematic clearing of sheltering shrubs from certain known breeding areas before the fly has had time to increase again in numbers. These measures would entail a relatively small expenditure of labour. The Islands of Nsazi, Lwagi and Bulago, altogether about eight square miles in area, could thus be easily cleared and these islands are quite suitable for cattle. The fly in some islands was tested by FRASER and the author in 1911 and proved negative to *T. gambiense*. CARPENTER found the Nsazi fly infective to monkeys in 1914. As regards the trypanosome reservoir, however, none of these islands present a serious difficulty. If it were possible to raise the level of Victoria Nyanza by some engineering device it would be possible to eliminate *G. palpalis* from the Lake. As there is only one outlet to the Lake, viz., at Ripon Falls, Jinja, which are 300 yards wide and divided into three parts by two islets, this could be accomplished by the erection of a permanent dam.

DU TOIT (P. J.). **Experimentelle Studien über die Pferdepiroplasmose. I. Mitteilung. Kreuzimfungsversuche mit *Nuttallia equi* (Laveran, 1901) und *Piroplasma caballi* (Nuttall, 1910).** [Experimental Studies on Equine Piroplasmosis. First Report. Cross Inoculation Tests with *Nuttallia equi* and *Piroplasma caballi*.]—*Arch. f. Schiffs- u. Trop.-Hyg.* 1919. May. Vol. 23. No. 7. pp. 121–135. With 4 charts.

Equine piroplasmosis is set up by two distinct causal organisms, that is, the term denotes in reality two separate diseases, which can

be differentiated clinically and epizootologically. A certain diagnosis can, however, only be established by a microscopic examination of the blood. Whereas *N. equi* exhibits itself as small round, oval, or pear-shaped intra-corpuscular parasites which multiply by each dividing into four separate elements, *P. caballi* takes the form of large pear-shaped organisms which are usually arranged in pairs within a corpuscle and divide by a peculiar process of budding. Mixed infections with both forms appear to occur but they must be of somewhat rare occurrence. Although the microscopic appearance of these two parasites, it is stated, are so distinct a modern author (SCHILLING, C., 1917) has dissented from the view that they are separate parasites and hence it became necessary to employ further methods for studying the characteristics of the organisms.



In this connection the author endeavoured to employ the so-called cross-inoculation or cross-immunity method first introduced by LAVERAN and MESNIL for the differentiation of various strains of trypanosomes, a method to which considerable importance has been attributed of recent years.

The application of the method for the differentiation of the piroplasmoses would, however, have to be carried out under circumstances markedly different from those employed in the case of experiments on the trypanosomiases. In the first place the piroplasms, as opposed to the trypanosomes, are strictly specific, and, secondly, a complete recovery does not take place subsequent to a piroplasm infection, for the infected animals remain carriers for the remainder of their lives (labile infection), and thus an animal cannot be "immunised," in the strictest sense of the word against piroplasmosis. Further,

in most of the domesticated animals one species of piroplasm only occurs so that no cross immunity test with a second species can be performed on these animals; horses, however, are an exception to this last rule inasmuch as they may be carriers of two species.

Two instances only of cross-innoculation with equine piroplasmosis appear to be on record in the literature. NUTTALL and STRICKLAND (1912) inoculated a horse that had withstood a light infection with *P. caballi* with *H. equi*. The animal became seriously ill and died after 20 days [see this *Bulletin*, Vol. 1, No. 1, p. 4].

CARPANO (1914) reported a similar case. A horse was first submitted to a slight infection with *P. caballi* and then was inoculated with *N. equi*; it became seriously ill but recovered. CARPANO'S experiment cannot be held to be conclusive inasmuch as this author worked with a horse that had already suffered from a piroplasm infection.

The author then describes in detail a series of eight cross-immunity experiments utilising blood obtained from Macedonia for infection with *N. equi* and blood from Roumania for infection with *P. caballi*. The results of these experiments are very briefly given as follows:—

The diagram on the preceding page also serves to illustrate the results obtained.

A horse that had withstood an infection with *P. caballi* was found to be resistent towards super-infection with the same organism—it was "immune." (Expt. 1).

A similar result was obtained in the case of a horse rendered "immune" towards *N. equi* and subsequently submitted to re-infection with the same organism. (Expt. 2).

Likewise so-called recurrent strains of the same causal organism failed to produce infection when inoculated into a horse rendered "immune" against the original strain. (Expt. 1*a* and 2*a* to 2*e*). These so-called recurrent strains were those piroplasms appearing in the blood during the subsequent febrile crises.

After super-infection with one strain of piroplasm a sudden rise of temperature may be observed, due apparently to a simple pyrogenic action of the parasite (inoculation reaction). (Expt. 1).

"Immunity" against *P. caballi* affords no protection against *N. equi*. (Expt. 3).

When a horse rendered "immune" against *P. caballi* is inoculated with *N. equi* the latter parasite only subsequently appears in its blood. (Expt. 3).

In such a case *N. equi* appears to disperse *P. caballi* completely from the blood. (Expt. 4).

Even after the termination of an acute *N. equi* infection *P. caballi* seems to disappear from the blood. (Expt. 8).

"Immunity" against *N. equi* does not protect against an infection with *P. caballi*. (Expt. 5 and 6).

If a horse "immunised" against *N. equi* is infected with *P. caballi* the latter parasite only appears in the blood. (Expt. 6).

The displacement of *N. equi* by *P. caballi* is, however, only apparent. The blood contains the former parasite even during the evolution of *P. caballi* infection in an invisible form. (Expt. 7).

The above experiments thus furnish decisive evidence in favour of the duality of the causal organisms of equine piroplasmosis.

The blood of a horse remains infective for a period of at least 14 months after the original infection (with *N. equi*).

The blood of a horse infected with *P. caballi* proved to be infectious even before the termination of the incubative period.

DU TOIT (P. J.). **Experimentelle Studien über die Pferdepiroplasmose. II. Mitteilung. Uebertragungsversuche mit *Ixodes ricinus* L. bei der *Nuttallia-equi*-Infektion.** [Experimental Studies on Equine Piroplasmosis. Second Report. Experiments upon the Transmissibility of Infection due to *N. equi* by *Ixodes ricinus*.]—*Arch. f. Schiffs-u.-Trop.-Hyg.* 1919. May. Vol. 23. No. 8. pp. 141–147. With 2 text-figs

During the War a number of German horses contracted biliary fever or piroplasmosis in the South Eastern theatre of operations and the question of the return of such horses into Germany presented considerable importance inasmuch as they might be regarded as being capable of spreading the disease either through the agency of infected ticks carried back on their bodies or by causing the infection to be taken up by the native tick *Ixodes ricinus* L., which is found almost universally throughout Germany. The possibility of the disease being spread by the first means would be very small but the problem as to whether the common German tick could act as a carrier, on the other hand, would merit careful study. Inasmuch as this tick is an intermittent feeder a number of experiments had to be performed in order to elucidate this point.

1.	Ticks infected as	adults	25/25 : 6 : 17	were allowed to feed on a susceptible horse in the subsequent	larval stage .. 3 : 11 : 17
2.		adults	25/26 : 6 : 17		nymphal stage. 24 : 4 : 18
3.		adults	25/26 : 6 : 17		adult stage .. 11 : 7 : 18
4.		larvae	27 : 6 : 17		nymphal stage. 3 : 11 : 17
5.		nymphs	4/5 : 6 : 18		adult stage .. 2 : 8 : 18

The results of the above experiments were invariably negative, from which one must conclude that *Ixodes ricinus* is not capable of transmitting *Nuttallia equi*. The author desires, however, that attention should be drawn to the fact that in some of his experiments the ticks did not bite well. It is also known that not all ticks become infected. It thus becomes possible that if some of these experiments were repeated with a larger number of ticks positive results might be obtained, although apparently the possibility is very remote.

The return of horses infected with *N. equi* into Germany thus offers no danger to the equine population if these horses are freed from ticks before they arrive. Inasmuch as the disease is naturally transmitted only by the agency of certain species of ticks and these are altogether absent in Germany the disease must die out with the death of the infected animals.

DU TOIT (P. J.). **Zur Systematik der Piroplasmen.** [The Classification of the Piroplasms.]—*Arch. f. Protistenk.* 1918. Vol. 39. No. 1. pp. 84–104. With 10 text-figs.

Up to about ten years ago all the blood parasites known as piroplasms were placed in the same Genus Babesia or Piroplasma and thus one reckoned as species of the same genus two organisms such as the parasite of Texas Fever (*Piroplasma bigeminum*) and the parasite of East Coast Fever (*Theileria parva*) which morphologically presented very little resemblance with each other. Since BETTENCOURT, FRANÇA and BORGES in 1907 separated the Genus Theileria from the Genus Piroplasma the piroplasms have been still further

classified until they contain at present at least seven genera. Four of these genera, however, each contain only one or two species; the majority of the numerous species of piroplasms known are contained in the genera Babesia, Theileria, and Nuttallia. However, it seems that authors are by no means agreed upon the characteristics which separately distinguish these species; for example, the bovine parasite first described by THEILER (1906) under the name *Piroplasma mutans* was placed by FRANÇA (1909) on morphological grounds in the genus Theileria. Inasmuch as the typical species of the genus Theileria—*T. parva*—undergoes a quite different development from "*T.*" *mutans* many authors do not share FRANÇA's views and place this parasite in the genus Babesia or Piroplasma. However, in the development of "*B.*" *mutans* one encounters "Maltese cross" forms, which are said to be characteristic of the genus Nuttallia; thus THEILER, GRAY and POWER (1914) emit the opinion that the parasite probably belongs to the last-named genus. On closer examination of the characteristics of this parasite one finds that it does not in reality fall into line with those described as being typical of any of the above three groups, and thus the author proposes the creation of a new genus, *Gonderia*, (in honour of the late distinguished protozoologist Richard GONDER) to include parasites of the *mutans* type. The author then gives a succinct historical review and analysis of the morphological and biological characters of the various known forms of piroplasms, and upon these grounds proposes the following classification.

1. Family: BABESIDAE.

Intracorpuscular blood parasites which do not contain pigment at any stage of their development and are transmitted by ticks of the sub-family Ixodinae. Multiplication in the circulation of the vertebrate host by division into two or four elements.

1. Genus: **Babesia** Starcovici, 1893.

Parasites which in one stage of their development are pear-shaped in form and which are disposed in pairs in the same blood corpuscle. Multiplication by division into two daughter parasites.

1*a*. Sub-genus **Babesia** (Starcovici, 1893).

Comparatively small parasites (on an average under 2·5 long), irregular in shape. Typical paired pear-shaped forms rarely seen. Division into two daughter parasites takes place by simple fission.

Babesia bovis	(Babès, 1888)	Ox
B. ovis	(Babès, 1892)	Sheep
B. argentina	(Lignières, 1903)	Ox
B. muris	(Fantham, 1906)	*Mus rattus* var. *albinos*
B. gibsoni	(Patton, 1910)	Dog and *Canis aureus*
B. divergens	(McFadyean & Stockman, 1911)	Ox
B. soricis	(Christophers ?)	*Sorex sp.*

1*b*. Sub-genus **Piroplasma** (Patton, 1895).

Comparatively large parasites (on an average over 3μ in length) with two nuclei, one large and vacuolated and one smaller and compact. The typical form is the double pear-shaped form. Division into two daughter parasites takes place as a rule by budding (as figured by NUTTALL and GRAHAM SMITH [1908] in the case of *P. bigeminum*).

Piroplasma bigeminum	(Smith & Kilborne, 1893)	Ox
P. canis	Piana & Galli-Valerio, 1895	Dog
P. pitheci	P. H. Ross, 1905	*Cercopithecus*
P. avicularis ?	(Wenyon, 1908)	*Avicularis zebrae*

P. leporis ?	Dschunkowsky & Luhs, 1909	Hare
P. caballi	Nuttall, 1910	Horse
P. trautmanni	(Knuth & Du Toit, unpublished)	Pig

2. Genus : **Nicollia** Nuttall, 1908.

Oval or pear-shaped parasites with a distinctly double nucleus (one large and one small karyosome). The daughter elements after division assume the form of a " clover leaf segmented into four parts."

Nicollia quadrigemina	(Nicolle, 1907)	*Ctenodactylus gondi*

3. Genus : **Nuttallia** França, 1909.

Oval or pear-shaped parasites. Multiplication by division into four daughter elements assuming the form of a Maltese cross. No typical rod-shaped parasites.

Nuttallia equi	(Laveran, 1901)	Horse
N. sp. ?	(Denier, 1907)	*Cervus aristotelis*
N. herpestedis	(França, 1908)	*Herpestes ichneumon*
N. sp. ?	(Patton, 1909)	*Herpestes mungo*
N. ninensis	(Yakimoff, 1909)	*Erinaceus europaeus*
= *N. veissi*	(Galli-Valerio, 1911)	*Erinaceus algirus*
N. sp. ?	(Dschunkowsky & Luhs, 1909)	Sheep in Transcaucasia
N. asini	(Dschunkowsky & Luhs, 1913)	Ass
N. sp. ?	(Dschunkowsky & Luhs, 1913)	Horse in Transcaucasia
N. muris	(Coles, 1914)	*Mus sylvaticus*
N. microti	(Coles, 1914)	*Microtus amphibius*
N. decumani	Macfie, 1915	*Mus decumanus*

4. Genus : **Smithia** França, 1909.

Parasites which in one stage of their development assume a pear-shaped form. The majority of these parasites occupy the whole diameter of the blood corpuscle and they do not divide by division into pairs. Multiplication takes place by division into four daughter elements arranged in the form of a cross.

Smithia microti	Franca, 1909	*Microtus incertus*
Sm. talpae	Galli-Valerio, 1913	*Talpa europaea*

5. Genus : **Rossiella** Nuttall, 1912.

Large parasites rounded in shape found singly, in pairs, or in fours in a single blood corpuscle. Nucleus large and round. Multiplication by division into two daughter elements.

Rossiella rossi	(Nuttall, 1910)	*Canis adustus*

6. Genus : **Gonderia** nov. gen.

Small round or rod-shaped parasites, multiplication by division into four daughter elements in the form of a cross, the products of division being composed almost entirely of chromatin.

Gonderia mutans	(Theiler, 1906)	Ox
G. dama ?	(Bettencourt, França & Borges, 1907)	*Cervus dama*
G. sp. ?	(Bettencourt & Borges, 1909)	*Cephalopus grimmi*
G. hirci	(Dschunkowsky & Luhs, 1909)	Goat
G. sp. ?	(Kerzelli, 1909)	*Rangifer tarandus*
G. cellii	(Castellani & Chalmers, 1910)	*Maracus pileatus*
G. hippotragi	(Todd & Wolbach, 1912)	*Hippotragus equinus*
G. buffeli	(Neveu-Lemaire, 1912)	Buffalo
G. sp. ?	(Carpano, 1913)	*Gazella sp.*
G. stordii ?	(França, 1913)	*Gazella granti*
G. sp. ?	(Rodhain, 1916)	Sheep in the Congo

G. brimonti	(Leger & Mouzels, 1917)	*Bradypus tridactylus*
G. camelensis	(Yakimoff & Schokhor, 1917)	Camel
G. rossica	(Yakimoff & Saphrono-witsch, 1917)	Field mouse

2. Family: THEILERIDAE.

Intracorpuscular pigment-free blood parasites, transmitted by ticks of the sub-family *Ixodinae*. Multiplication takes place principally by schizogony in the tissue cells of the vertebrate host.

1. Genus: **Theileria** Bettencourt, França & Borges, 1907.
Small round or rod-shaped parasites. Multiplication by simple binary division or by schizogony in the cells of the lymphatic system.

Theileria parva	(Theiler, 1904)	Ox
Th. annulata	(Dschunkowsky, 1904)	Ox
Th. sp. ?	Lichtenheld, 1911	Antelope
Th. ovis	Littlewood, 1914	Sheep
Th. tachyglossi	Priestley, 1915	*Tachyglossus aculeatus*

2. Genus: **Rangelia** Carini & Maciel, 1914.
Round, oval, or pear-shaped parasites. Multiplication by binary division or by schizogony within the connective tissue or endothelial cells.
Rangelia vitalii (Rangel Pestana, 1910) Dog.

The author then appends some remarks upon his system of classification. OLLWIG and MANTEUFEL (1912) had already indicated that the piroplasms necessitated re-grouping into more than one family on account of the different phenomena observed in a study of their life cycle. The two separate families proposed by the author above show few characters that are in common. Hitherto the piroplasms were usually regarded as members of the Haemosporidia or as a set of organisms nearly related to this order. In the light of recent knowledge this view can no longer be held. If the observations of some authors (BREINL and HINDLE, KINOSHITA and others) with regard to the presence of flagellate stages in piroplasms (*P. canis*) are confirmed then these organisms become nearly related to the Flagellata. In view of the results of recent researches in this connection (especially those of PASCHER, 1917) the expression "flagellates without flagellum" is by no means a contradiction in terms, for the absence of flagella in piroplasms during their intra-cellular existence becomes quite comprehensible. If one allows HARTMANN'S proposed Order Binucleata to stand then the piroplasms phylogenetically fall undoubtedly into this group. Many piroplasms (Piroplasma, Nicollia, Babesia, etc.) present the greatest morphological similarity with Leishmania, and the development of Theileria is, according to the researches of HARTMANN (1917) and others, almost identical with that of Schizotrypanum and Haemoproteus, which, it is stated, are typical flagellates.

The genus Babesia is divided into two sub-genera inasmuch as the differences presented by representatives of these two groups were not deemed to be of anything like the same importance as those separating the various orders of the Babesidae.

The author then further elaborates the differences which in his mind renders necessary the separation of the piroplasms into the above genera and species. There are still a few organisms which do not fall exactly into any of the above divisions.

In a postscript the author criticises the system of classification suggested by FRANÇA [see this *Bulletin*, 1917, Vol. 5, No. 4, p. 231].

This author regarded the piroplasms (Piroplasmidae) as a Family of the Sub-Order Haemosporidia which with the Coccidia formed the Order Coccidiomorpha, which with the Gregarina formed the sub-class Telosporidia; these again, with the Neosporidia, forming the Class Sporozoa. Du Toit has already adduced reasons for regarding the piroplasms as being probably members of the Flagellata and not of the Sporozoa in view of recent researches. FRANÇA further sub-divided the Family into the following Genera, *Piroplasma*, *Smithia*, *Nuttallia*, *Theileria*, *Paraplasma*, *Achromaticus*, *Rangelia*, *Rossiella*, and *Elleipsisoma*.

VELU (H.). **La Coccidiose de la Chèvre au Maroc et le Parasitisme latent de *Eimeria arloingi*.** [Coccidiosis of the Goat in Morocco and Latent Parasitism of *Eimeria arloingi*.]—*Bull. Soc. Path. Exot.* 1919. June. Vol. 12. No. 6. pp. 298–301.

Coccidiosis was first identified in goats by MAROTEL (G.) in 1905, and since then the subject appears to have been very little studied. MARTIN (P.) of Dresden (1907) gave a description of the intestinal lesions. STEVENSON (1909) observed several cases at the Wellcome Research Laboratories, Khartoum, the results of his examinations being published in the Fourth Annual Report of the Laboratories in 1911. MARTIN (A.) of Toulouse (1912) gave an account of the histology of the lesions found in an affected kid from Tunis. BALFOUR (A.) observed two cases in the course of his expedition up the White Nile.

The author in this note gives an account of a veritable outbreak of coccidiosis in a herd of 82 Spanish goats and their 60 offspring imported into Casablanca. The causal organism was identical with that fully described by MAROTEL and named by him *Eimeria arloingi*.

The disease manifested itself exclusively as an affection of very young kids, only a few weeks old and still sucking. This fact led the author to believe at first that the outbreak consisted of numerous cases of navel ill. Fatalities occurred only in these young animals—50 (or 25 per cent.) died during the month of March and the beginning of April after having shown very acute symptoms. In the case of the remaining 45 no noteworthy symptom was observed.

The disease ran a very rapid course characterised by progressive anaemia with intestinal derangement. The animals first became affected with loss of condition, then dullness, and loss of appetite, and remained lying down. The faeces were at first normal; then diarrhoea set in which subsequently became mucous or serous in character. In the mucous discharge coccidia were extremely numerous and at a more or less advanced stage of their development. Death usually occurred after a week although sometimes it took place more slowly. For two or three days preceding death the affected animals were incapable of getting up and sometimes showed convulsive crises. Cutaneous lesions as described by STEVENSON and BALFOUR were not observed.

The lesions are said to be quite characteristic and to resemble those found in intestinal coccidiosis of lambs.

The author succeeded in setting up the disease experimentally by feeding two healthy Moroccan he-goats on fresh infected faeces.

Ten days after having been thus fed their faeces showed slight alterations. Instead of being expelled in the form of hard separate well moulded pellets they took the form of a cylindrical mass formed by the union of somewhat softer pellets adherent to each other by means of a thin mucous covering. Coccidia were relatively numerous in this material, being easily discoverable by the ordinary methods of concentration. The characters of the faeces were the same a month later and coccidia were also numerous; however, the goats showed no sign of disease. The faeces would have shown no appreciable alteration to an unsuspecting observer. These two animals infected experimentally might thus be considered latent carriers of coccidia capable of spreading the parasite by means of their faeces and of thus propagating the disease to more susceptible animals.

Examination of the infected herd showed that this view with regard to latent parasitism was correct. Among the adult animals there was no visible sign of disease although the dams furnished a somewhat smaller quantity of milk than normally; however, examination of the faeces demonstrated the presence of coccidia in every case, the entire herd being infected. It was thus evident that the adult animals regarded as healthy had infected the young ones.

In the course of subsequent investigations the author discovered the presence of *Eimeria zurni* and its latent parasitism among bovines in Chaouia.

Several methods of treatment are said to have been adopted in the case of the affected and apparently healthy animals, without, however, obtaining the least beneficial result. Emetine hydrochloride was inefficacious. In the hands of Italian veterinarians thymol appears to have given surprisingly good results in the treatment of bovine coccidiosis; however, it produced no effect when given by the author in doses of 3 grammes repeated three days in succession, and administered in 1 gramme powders at hourly intervals. On the other hand the disease rapidly disappeared and the adult animals acquired quite a satisfactory condition after the herd had been moved on to a fresh sandy soil situated near the seashore.

LAVERAN (A.) & FRANCHINI (G.). **Au sujet de l'*Herpetomonas ctenocephali* de la puce du chien et de sa culture.** [*H.c.* of the Dog-flea and its Cultivation.]—*Bull. Soc. Path. Exot.* 1919. June. Vol. 12. No. 6. pp. 310–313. With 2 text-figs.

In 1915 the authors described a herpetomonad from the mouse flea to which they gave the name *H. ctenopsyllae.* Pure cultures of this flagellate were obtained but unfortunately no further work could be performed with the organism until recently, when the investigations were continued with the herpetomonad *H. ctenocephali* from the dog flea. In 1914 WENYON obtained pure cultures of this organism. *A priori* it would seem to be very difficult to obtain pure cultures of living flagellates from the digestive tract of insects, that is, from a medium that is supposed to be rich in bacteria. This difficulty, however, appears to have been exaggerated, at least in so far as it concerns the flagellates from fleas. If one examines the contents of the digestive tract of a flea bacteria are often found to be present only in very small numbers. This might be explained by the fact

that the insects ingest a sterile food material, viz., blood. The contents of the intestine of fleas heavily infested with flagellates are particularly poor in bacteria. In this case the reaction of the digestive tract of the flea is neutral or slightly alkaline whereas in fleas not infected with flagellates and showing numerous bacteria in their intestine the reaction is slightly acid.

According to the authors' technique the flea caught is placed in a test tube and killed by means of ether vapour, after which it is washed very carefully by passing through seven or eight baths of sterile normal saline solution placed in watch glasses, all the instruments used being sterilised with care. The flea is then placed in a drop of citrated normal saline solution on a glass slide and dissected by means of fine mounted needles in such a manner as to isolate the digestive tube, from which the contents are extracted by pressing with the needle. One makes certain of the presence of the flagellates by examining a drop of liquid in the fresh state, and then by means of a fine pipette one or two tubes of simplified Novy medium are inoculated with the fluid. The culture tubes, which should have been recently prepared, are kept at a temperature of 24° to 25° C. The culture develops badly or even fails altogether in tubes that have been prepared for longer than a fortnight.

In two experiments out of four the attempts at culture of the flagellate from the dog flea succeeded. The organisms showed a close resemblance to those obtained from the mouse flea examined under similar conditions, the shape and size of the parasites were about the same. However, in the case of the organisms cultivated from the dog flea the majority possessed long flagella, which are absent or very short in those from the mouse flea. Further experiments are being performed in order to determine whether the dog flea flagellate is capable of being transmitted to mammals.

LAVERAN (A.) & FRANCHINI (G.). **Infection des souris blanches à l'aide des cultures de** *Herpetomonas ctenocephali.* [Infection of White Mice with Cultures of *H. c.*]—*Bull. Soc. Path. Exot.* 1919. July. Vol. 12. No. 7. pp. 379–383. With 3 text-figs.

In this note the authors state that the flagellates obtained in culture from the dog flea were easily sub-cultured in simplified NOVY medium (12 generations). In 1913 the authors showed that mice could be easily infected by inoculating them intraperitoneally with the contents of the digestive tract of dog fleas infested with the flagellates. It was interesting to know what results would be obtained by inoculating mice with pure cultures of these organisms. Eight mice were inoculated once to three times with cultures obtained in simplified Novy medium or with sub-cultures. In two cases the mice did not show any sign of infection after having been kept under observation for from one to two months. In four cases slight infections of short duration were noted. One mouse was sacrificed in very poor condition and another died as the result of the infection; a detailed account is given of the course of the infection in these two mice.

In the case of the first mouse the infection was manifested as a severe anaemia which would eventually have brought about a fatal termination if the mouse had not been killed 25 days after inoculation.

The parasites were then found to be fairly numerous in the blood, and still more numerous in the liver and the spleen. In the liver the parasites had invaded not only the leucocytes but also the hepatic cells and their leishmaniform aspect was remarkable. Blood from the heart of this mouse, sown in tubes of simplified Novy medium, gave rise to abundant cultures of flagellates identical with those obtained by sowing the medium with the contents of the digestive tract of the infected dog fleas. One has thus at hand an easy means of obtaining from invertebrates pure cultures of the flagellates which are inoculable into mammals.

In the second mouse referred to, the haematozoa although rare in the peripheral blood were fairly numerous in the liver and spleen. There seemed to be no doubt but that the infection set up by the flagellates was the cause of death.

CHATTON (Edouard). **Sur la culture pure d'un *Leptomonas* de la puce du chien et sur un caractère de ses formes culturales qui les distinguent de celles du Kala-Azar de souches humaine et canine.** [Pure Culture of a Leptomonas from the Dog-flea, and a Character of its Cultural Forms which Distinguishes it from the Human and Canine Strains of Kala Azar.]—*Bull. Soc. Path. Exot.* 1919. June. Vol. 12. No. 6. pp. 313–316.

In the course of some researches performed in connection with kala azar at the Pasteur Institute, Tunis, the author came into possession of a dog, the fleas from which were infected in the proportion of 75 per cent. with a Leptomonas. This infection was confined to the rectal sac which was filled with parasites ovoid in form, each with a flagellum transformed into a mucous thread, by means of which they adhered to the walls of the sac and to each other; that is, they presented a gregarian appearance. The rectal sac as a rule contained nothing else and in particular no bacteria. Inasmuch as the dog harbouring the fleas was to be inoculated with kala azar it was necessary to make sure that it was free from this infection; inoculation of media from the tibial bone marrow and from its blood gave no growth. Since numerous authors have imputed the transmission of Mediterranean kala-azar to fleas it was of interest to find out whether the flagellate from the dog flea could be identified with that of kala azar or, on the other hand, differentiated from it. Inoculation of material from 30 crushed fleas into two mice intraperitoneally gave no result. The blood of these mice sown on NNN medium 8 and 33 days after inoculation gave no growth. Post-mortem preparations—made after three months—showed no parasites.

The author endeavoured to isolate the organisms in culture inasmuch as they appeared to be in a state of purity in the rectal sac and had been cultivated successfully by other authors (WENYON 1914, NÖLLER 1917, and LAVERAN and FRANCHINI 1915). Two fleas were washed by shaking up well in three tubes successively of sterile normal saline solution. The fleas were dissected with aseptic precautions and the posterior part of the intestine was again washed three times in sterile saline solution. One of the sacs was opened by puncturing with a needle and placed in the condensation liquid of an NNN tube. The other was divided into small fragments and its contents suspended

in 5 c.c. of normal saline solution. Four tubes of NNN medium were inoculated with 10 drops, 5 drops, and a loopful, respectively, of this suspension.

Between the 8th and 15th day all the above tubes showed a pure culture of Leptomonas. At first the culture was similar to that of all cultures of Leishmania or Leptomonas but it soon became easy to recognise the presence of large monads in the form of large flagellated needle-shaped structures—the bodies of which were twisted in screw-like fashion exactly as in the case of *Leptomonas davidi* Lafont of the Euphorbias. These forms were to be found constantly even in primary culture. They appear to be noteworthy inasmuch as they enable one to distinguish readily and with certainty this Leptomonas culture from those of *Leishmania infantum* and *L. tropica*, which never show these corkscrew-like flagellates.

The author was able to compare the strains from the flea with cultures of kala azar of canine origin isolated by Charles NICOLLE from two dogs and after several months' observation these never showed the peculiar twisted bodies. This is important evidence against the suggestion of PATTON (1914), who was inclined to regard canine kala azar as being quite different from the human infection and to be set up by an organism identical with the Leptomonas from the dog flea. Chatton asserts that as the result of his cultural studies the Leptomonas isolated from the dog flea has nothing in common with the Leishmania of canine kala azar in Tunis. No mention has been made of forms analogous to those described in this note by other authors who have cultivated Leptomonas from fleas. It may be that Chatton was dealing with a new species. In the cultures there were also non-flagellated ovoid forms, more refractile *in vivo* than the other forms and which might perhaps be regarded as the equivalent of encysted forms. These were, however, very rare in the fleas.

CHATTON (Edouard) & BLANC (Georges). **Inoculations positives de cultures de *Leishmania tropica* aux Geckos.** [Positive Results of Inoculations of Cultures of *L. tropica* into Geckos.]—*Bull. Soc. Path. Exot.* 1919. June. Vol. 12. No. 6. pp. 316–322.

In this paper the author discusses the probability of certain lizards including the gecko being able to act as reservoirs of the virus of oriental boil; the results of other investigators are quoted and a record of the authors' own experimental work given. It appears that the gecko, *Tarentola mauritanica*, which is infected with leptomonads in Southern Tunisia to the extent of 37·5 per cent. is free from infection in the north of the Regency. It would be very interesting to map out the limits of the distribution of the flagellate. When cultures of *L. tropica* are inoculated intraperitoneally into the gecko the flagellates pass into the circulating blood from which they can be extracted by cultural methods for at least 13 days after inoculation. The presence of this parasite is not rendered evident by any symptom or any lesion in the blood or organs. This is also the case in natural infection of the gecko or after inoculation with Leptomonas. Neither Leishmania nor Leptomonas can be discovered by direct examination. It is only by culture in NNN medium that their presence can be discovered. Hitherto it has not been possible to reproduce oriental

boil in man or monkeys by means of Leishmania passed through geckos. It would be interesting to find out if there occurs a loss of virulence in this passage. *L. tropica* does not undergo, after passage through geckos, any change in the characters which enable one to distinguish it culturally from Leptomonas peculiar to the reptile.

Di Domizio (Giovanni). **Dell'*Anaplasma marginale*. (Corpi di Jolly nel sangue anemico. — Forme anaplasmatiche di piroplasmi.)** [*Anaplasma marginale*. Jolly Bodies in Anaemic Blood: Anaplasmatic Forms of Piroplasms.]—*Clinica Vet.* 1919. Apr. 15 & 30 & May 15-31. Vol. 42. Nos. 7–10. pp. 203–220, 237–251, & 292–311. With 2 text-figs. & 2 plates comprising 12 figs.

In this article the author passes under review all the literature relevant to the much disputed question as to the specificity of the so-called anaplasms or marginal points. These bodies, mainly as the result of the experiments and observations of Theiler in South Africa, Lignières in South America, and other workers in other parts of the world, have been incriminated as being the actual cause of serious disease, especially in cattle. On the other hand, other workers of repute have refused to admit the parasitic nature of these so-called marginal points but regard them either as disintegrated nuclear remains (Jolly bodies) often found in anaemic blood or as the degenerated protoplasm-free remains of certain blood parasites such as *Piroplasma bigeminum*. The author discusses the various conclusions arrived at by these workers at some length and adds a description of his own observations in this connection among the domesticated animals in the Italian colony of Eritrea. An account of some experimental work upon the changes brought about by the production of severe experimental anaemia in laboratory animals is also given, in order to compare the structures known as Jolly bodies found in anaemic blood with those which he regarded as true anaplasms, or bodies strictly parasitic as conceived by Theiler and Lignières, found in the blood of sheep and goats in Eritrea and considered to be new species of the genus Anaplasma.

Among other matters mentioned a detailed description is given of the discovery of marginal points by the author in a goat in 1913 in a district in northern Eritrea. Unfortunately experiments could not be performed upon animals. The most important observations concerning anaplasms in sheep and goats are:—(*a*) those of Schellhase (1912), who found these bodies either alone or together with piroplasms of the *mutans* type in "German" East Africa in sheep and goats affected with a somewhat ill-defined disease characterised by intermittent fever, severe and progressive cachexia, and purulent discharge from the eyes and nose; (*b*) of Trautmann (1913), who made similar observations upon sheep in "German" East Africa suffering from a disease showing a clinical form somewhat similar to the above; (*c*) of Bevan (1912), who found evidence of anaplasmosis in Rhodesia among sheep affected with helminthiasis, and subsequently stated that the disease was very widespread in that country.

The goat brought under the author's observation was reported to have been ill for about 10 days suffering from inappetence, progressive

loss of condition, and lachrymation, extreme pallor of the mucous membranes, slight fever (temperature 40·7° C.), and a weak rapid pulse. Microscopic examination of the blood showed severe anaemic changes and in particular a striking picture of "marginal points." On post-mortem examination a large caseating centre was found in one lung, extreme pallor of all the tissues, and watery blood and sero-gelatinous transudates were found in the abdomen, at the base of the heart, around the aorta and trachea, at the entrance to the chest under the loins, and around the intestines, etc. The liver was enlarged and degenerated, gall bladder distended with thick bile, spleen enlarged; the urine was of normal colour.

On microscopic examination after staining with Giemsa the blood showed some red corpuscles with large and small basophile granules while others were nucleated, together with polychromasia, poikylocytosis, anisocytosis, and leucocytosis. About one-half or more of the red corpuscles showed marginal points, the invaded corpuscles sometimes containing two or three bodies although the great majority only contained one. These were seen as minute more or less rounded or oval bodies from 0·4 to 1μ in diameter with indistinct, sometimes irregular, margins situated for the most part towards the periphery of the corpuscles, some causing a small peripheral protuberance; others were more or less adjacent to the margin of the corpuscles, whilst some appeared to be completely extra-corpuscular. A very small minority were situated towards the centre of the corpuscle. The bodies stained of a purplish red colour whilst towards their periphery a certain number showed appreciable signs of being less deeply stained, of almost a rose colour, and these forms showed on close examination a very minute sprouting process. Paired forms, like diplococci, were sometimes seen, one body being then usually two or three times the size of the other. In very rare cases of this sort an extremely fine chromatinic filament was seen to unite the two bodies when separated some distance from each other. In blood smears from this goat extremely rare piroplasms of the *bigeminum* type (*P. ovis*) were found.

The author next refers to the work of Dias and Aragao (1913), Laveran and Franchini, and Finzi and Campus [see this *Bulletin*, Vol. 5, No. 1, pp. 3–5] upon the production of a severe anaemia in rabbits and sheep by the subcutaneous injection of phenylhydrazine and nitrobenzol in order to bring about the appearance of bodies which they claimed to exhibit no visible differences from the so-called marginal points. Experiments in this direction were conducted by the author himself utilising four rabbits, two lambs, one goat, and one kid. Details are given concerning one rabbit, one lamb, and one goat. It was found that a few rare Jolly bodies were first manifested in the blood of the rabbit 24 to 48 hours, in the lamb three days, and in the goat two days after injection. These gradually increased in number and reached their maximum on the 6th and 8th days in the rabbit, on the 8th and 11th days in the lamb, and on the 12th and 14th days in the goat. They then gradually diminished in number. The maximum number of red corpuscles invaded with Jolly bodies in the blood of the rabbit was from 4 to 5 per cent., in the lamb 5 to 6 per cent., in the goat 4 to 5 per cent. The maximum in each animal coincided with a stage of manifest amelioration in the anaemic condition of the blood. In the other two surviving animals, a rabbit and a

lamb, treated with nitrobenzol and phenylhydrazine, the anaemic changes were of a similar nature and Jolly bodies appeared in the rabbit a little later than in the above case, and in the lamb the maximum, 4–5 per cent., was reached on the 10th and 12th days. These Jolly bodies within the red corpuscles were usually seen towards the periphery as small spherical-looking masses staining of a deep dark violet colour like the chromatin of the nuclei in normoblasts. In diameter they varied from 1 to 3μ and occurred singly in the great majority of cases although in a few corpuscles they were seen in pairs like diplococci of unequal dimensions. In rare instances three or four bodies of unequal dimensions were found in the same corpuscle and more or less attached to each other thus forming a mass resembling more or less the nuclei of normoblasts. Very rarely the bodies were seen as minute just visible dots. A few rare irregularly stained forms were also seen.

The nature of such bodies came also to be studied by the author in the course of anti-rinderpest inoculations in Eritrea (1918). A protective serum was obtained from one animal with very marked haemolytic properties. Sucking calves died in from 12 to 48 hours after injection with serum from this animal with severe symptoms of haemolysis; young and adult animals showed for some time only a more or less distinct pallor of the mucous membranes. Microscopic examination was made of the blood from about 15 of the fatal cases and from another 15 cases once or twice daily for a period of 20 days. The disease was further studied by injecting four calves with a dose of serum which was not excessive (15 to 20 c.c.). Signs of more or less severe haemolysis were first seen in these four animals in from 8 to 10 hours and smears were made systematically from the peripheral blood during the following fortnight. The presence of Jolly bodies, however, on no occasion was a striking character of the anaemia and the percentage of red corpuscles containing these bodies did not exceed 3 to 4 per cent. The author thus maintains that there is a striking difference between "marginal points" and Jolly bodies both with regard to their morphological characters, staining reactions, percentage of red corpuscles invaded, and their disposition within the corpuscles, points which are dealt with at considerable length.

However, the question still remains as to whether the so-called anaplasms or "marginal points" are identical with degenerating forms of piroplasms. No direct experimental evidence is adduced to prove that these bodies are in reality dissimilar but from a critical examination of the known facts and especially on analogy with the results of THEILER's and LIGNIÈRES's experiments in connection with *Anaplasma marginale*, or *argentinum*, and *Piroplasma bigeminum* the author claims that there are sufficient grounds for concluding that the bodies discovered by him are in the nature of true parasites—*Anaplasma ovis*.

He concludes as follows:—

"(1) There exists among goats in the colony of Eritrea an intracorpuscular blood parasite which in all probability represents a species belonging to the genus Anaplasma (*Anaplasma ovis*).

"(2) The researches and observations, upon which various authors have based doubts upon the parasitic nature of anaplasms—have maintained that they were nuclear fragments (Jolly bodies) or, on the other hand, stages in the life cycle of some piroplasms—do not detract from the

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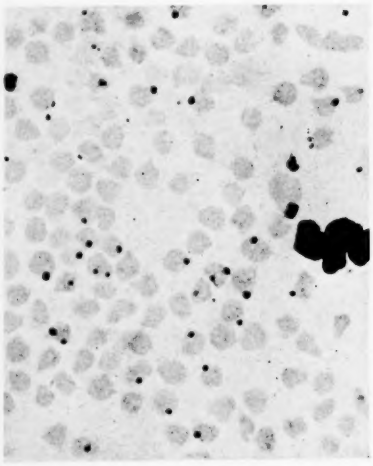

Fig. 1.

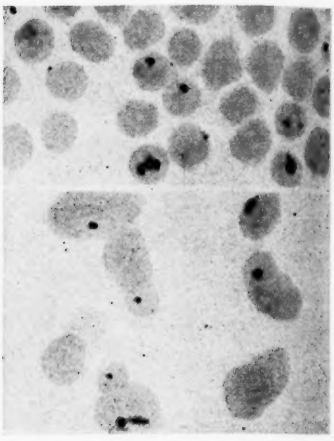

Fig. 4.

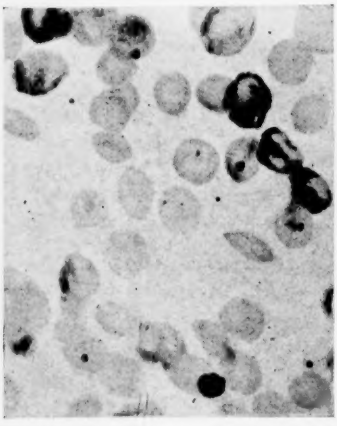

Fig. 7.

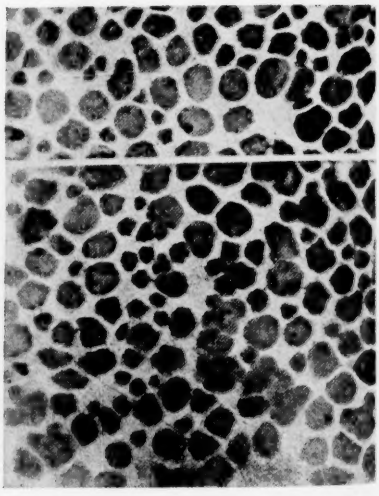

Fig. 11.

Fig. 1. Smear from the peripheral blood of the Eritrean goat with large number of corpuscles infected with marginal points (*Anaplasma ovis*).

Fig. 4. Smears of the peripheral blood of a horse affected with nuttalliasis. Anaplasma-like form of *Nuttallia equi*.

Fig. 7. Smear of peripheral blood from a young dog, belonging to a European breed, born in Eritrea. Corpuscles containing Jolly bodies.

Fig. 11. Smear from a goat suffering from marked anaemia as the result of inoculation with nitrobenzol and phenyl hydrazine, blood defibrinated and kept in a test tube at a temperature of 25°C. Kept in tube for 8 hours before smears were made. Red corpuscles with Jolly bodies.

[Reproduced from *La Clinica Veterinaria*.]

value of THEILER'S and LIGNIÈRES'S experiments, which demonstrated the existence of a new genus of parasite named by THEILER Anaplasma.

"The morphology described as characteristic of *Anaplasma marginale* (and its variety *centrale*) and of *Anaplasma argentinum*, as described by THEILER and LIGNIÈRES, is distinguishable from that of the Jolly bodies found in anaemic blood; the considerably higher percentage of the red corpuscles invaded with marginal points is of especial importance in this connection.

"These anaplasms are also distinguished from the so-called anaplasm-like forms of piroplasms inasmuch as a greater percentage of corpuscles is invaded with marginal points and these points are quite spherical in form and each has sharply defined borders.

"(3) Some authors in descriptions of alleged marginal points, examined singly, have pictured them as having irregular and indistinct borders (like those of the anaplasma-like forms of piroplasms) but the general aspect of true anaplasms leaves no room for doubt.

"Although Anaplasma has only been observed as a well-defined entity among bovines in South Africa and Argentina the collective observations and researches of many authors appear to show that (*a*) these bodies exist not only in bovines but also in other animals, (*b*) very probably there exist several species or varieties of anaplasms not only in various species of animals but also in the same species of animal, (*c*) these various species or varieties, although conforming morphologically with typical Anaplasma, present slight differences in form."

[Four figures are reproduced from the plates in order to show how difficult it must be, in spite of the author's contentions, to form a definite opinion with regard to the nature of anaplasms from their morphology alone.—ED.]

NOGUCHI (Hideyo). **Etiology of Yellow Fever.**—*Jl. Exper. Med.* 1919. June. Vol. 29. No. 6. pp. 547–596. With 8 plates.

In this series of three contributions Noguchi gives an account of his investigations upon the etiology of yellow fever, the material for study being obtained from cases of the disease admitted to the Yellow Fever Hospital in Guayaquil, Ecuador. In the first paper an analysis is given of the symptomatology and pathological findings in 172 cases observed, but from this study no facts are said to have been brought to light which had not been described by previous students of yellow fever.

In the second paper the author describes some transmission experiments with the disease. It was found that by injecting into guinea-pigs the blood of yellow fever cases a group of symptoms and lesions closely resembling those observed in the human disease were induced in a limited number of cases. Of 74 guinea-pigs inoculated with specimens of blood from 27 cases of yellow fever, 8, representing 6 cases, subsequently exhibited the symptoms: viz., a marked rise of temperature after a period of incubation lasting on an average from 3–6 days, with simultaneous suffusion of the capillaries, particularly of the conjunctiva and the soles, then preliminary hyper-leucocytosis followed by progressive leucopenia, the early appearance of albumen and casts in the urine, which gradually diminished in volume as the disease progressed. The fever lasted only a few days, the temperature rapidly dropping first to the normal level and then usually becoming sub-normal. At this period jaundice manifested itself in various degrees of intensity first in the scleras, then in the skin and the urine. Haemorrhages from the mucous membranes of the nostrils or gums or from the anus were observed to occur during this period. On post-mortem examination intense jaundice was revealed throughout

all the tissues. The liver was fatty and yellow, the kidney hyperaemic and often swollen and haemorrhagic. Haemorrhagic spots were almost always found in the lungs and gastro-intestinal mucous membrane. Guinea-pigs were usually rather sensitive to the infection though many appeared to be somewhat resistent and some even refractory.

The injection of the yellow fever blood into ring-tailed monkeys, rabbits, cats, guatusas, weasels and sloths among mammals, and pigeons, ground doves, blue birds, mantas, blackbirds, parrakeets, reed birds, blancos, and toucans among birds, gave negative results.

In the blood, liver, and kidneys of the guinea-pigs experimentally infected a minute organism was demonstrated which closely resembled in morphology the causative agent of infectious jaundice in man (*Leptospira icterohaemorrhagiae*). The leptospira transmitted from yellow fever cases to guinea-pigs was found to induce similar symptoms and lesions upon further passage into normal guinea-pigs. The author gives the provisional name of *Leptospira icteroides* to this organism.

In the third paper Noguchi discusses the symptomatology and pathological findings in animals experimentally infected; the mode and course of the infection as observed in guinea-pigs, dogs, and monkeys is described. Infection with the organism, it is stated, can be induced either by infection into the peritoneal cavity, the blood circulation, or the subcutaneous tissues, or by application to the scarified depilated surface of the skin or to the mucous membranes, or by feeding the animal with infected tissue or culture.

The symptoms and lesions induced in guinea-pigs were much more pronounced than those observed in dogs or the marmoset. The period of incubation was nearly the same in all three species, 72–96 hours in the case of intraperitoneal or subcutaneous inoculation and a day or two longer when the infection was induced percutaneously or *per os*. The febrile reaction in the guinea-pig and marmoset was about the same. In the dog there was less fever. The amount of albumen, casts, and bile pigments in the urine was more abundant in the guinea-pig and marmoset than in the dog, and these animals also appeared on the whole to become more intensely jaundiced. A black or bile-stained vomit, however, though occurring frequently in the dog during life was observed in the guinea-pig and marmoset post-mortem. Petechial lesions were most pronounced in guinea-pigs, less marked in marmosets, and least in dogs. In dogs, for example, subcutaneous haemorrhages hardly ever occurred and the lungs usually only showed a few minute ecchymoses; the pleurae, pericardium, and other serous surfaces of the thorax and abdomen remained free from ecchymoses which, however, with hyperaemia were very marked along the gastro-intestinal tract. The symptoms and lesions thus observed in animals experimentally infected with *Leptospira icteroides*, whether obtained from the blood or organ emulsions of guinea-pigs or other susceptible animals experimentally infected or from a pure culture of the organisms, were closely parallel to those of human yellow fever. The pathological changes occurring in human cases are similar to those induced by inoculation in guinea-pigs and marmosets and in respect of their intensity stand intermediate between those arising in these two animals.

DISEASES DUE TO FILTERABLE VIRUSES.

REMLINGER (P.). **Comment un chien d'apparence saine peut transmettre la rage.** [How an Apparently Healthy Dog may transmit Rabies.]—*Bull. Soc. Cent. Méd. Vét.* 1919. May 15. *Rec. Méd. Vét.* 1919. May 30–June 30. Vol. 95. Nos. 10 and 12. pp. 175–181.

In 1907 the author issued a publication discussing the possibility of the transmission of rabies by healthy dogs. He reported a case of a young girl that had come from Smyrna to the Anti-Rabic Institute of Constantinople as she feared infection from her young sister, dead of rabies a few days previously. It was stated that this child had not undergone the Pasteur treatment inasmuch as the dog that had bitten her in the foot five months previously showed no suspicious symptoms and was then alive and healthy. In the medical literature a certain number of analogous cases are described and in this paper the author discusses the pathogenesis of such cases, which he admits are very exceptional and more interesting from a scientific than from a practical point of view. In the first place it has to be acknowledged, for various reasons which are enumerated, that all these cases of rabies contracted by the bite of a healthy dog are not beyond criticism. BABÈS was of opinion that these cases of transmission by apparently healthy dogs could be explained by the fact that the animals were suffering from abortive manifestations of the disease such as fever, loss of weight, and irritability, which were quite curable. It seems, however, from the experimental investigations of KONRADI [see this *Bulletin*, Vol. 2, No. 2, p. 94; Vol. 4, No. 2, p. 62; and Vol. 5, No. 2, p. 118], and the author himself [see this *Bulletin*, Vol. 6, No. 3, p. 183] on the hereditary transmission of rabies that a somewhat different solution of the problem can be offered. The transmission of rabies from mother to foetus can be observed to take place in the following conditions.

(1) On post-mortem examination of an animal dead of rabies one may find foetuses at a more or less advanced stage of development; inoculation of the brain of these foetuses into guinea-pigs sets up rabies.

(2) A very few days before dying of rabies an animal gives birth to quite healthy-looking young, which, however, at a more or less remote date suffer from the disease and succumb.

(3) Some time after having been inoculated with the virus an animal may give birth to progeny, apparently perfectly developed and which may live for several weeks or even months without showing commencing symptoms of rabies, from which they succumb. The mother still remains alive and in perfect condition and only dies from rabies some considerable time after its young. This class of hereditary transmission is considered worthy of minute examination. The facts in connection with this particular mode of transmission were first studied by KONRADI and in this paper the author describes some experiments which confirm his conclusions.

A guinea-pig inoculated by the author into the foot with "street" virus gave birth 50 days subsequently to three young. One of these

succumbed when 13 days old and apparently rabies was not the cause of its death. The other two succumbed at the age of 34 days, showing very suspicious paralytic symptoms and transmission experiments showed that they had in fact died from rabies. The mother survived its three young and only showed symptoms 38 days after the death of the last two, and it died from rabies on the day following first symptoms.

It thus appears that rabbits and guinea-pigs are capable of transmitting rabies *in utero* to their young whilst themselves showing all the signs of good health. They may even transmit rabies sometimes for a period of more than a year before succumbing to the disease. In the case of the dog KONRADI observed a fact of somewhat similar nature. A bitch inoculated showed commencing symptoms of rabies 11 days after parturition and the disease made its appearance in its six young 11, 43, 61, 66, 68, and 69 days, respectively, after birth. There is thus no reason to suppose that the dog behaves in a manner different to the guinea-pig and rabbit and KONRADI rightly insists upon stating that in the matter of the transmission of rabies from mother to foetus there appears to be no difference among the various species of animals. Inasmuch as these experiments were performed with "street" virus it seems justifiable to conclude that the same results are obtained in nature.

ROUX and NOCARD showed that two or three days before the appearance of any apparent change in the demeanour of an infected dog its saliva had become virulent. Various other authors have claimed to have established that the saliva is infective for even a longer period prior to the onset of symptoms (KONRADI, 14 days). If indeed the rabies virus is discoverable in the blood several months before death there is no reason to doubt but that it may also be found during the same period in the saliva. BERTARELLI claims to have shown that the virus gains access to the salivary glands by travelling along the peripheral nerves and not by way of the blood stream. The author himself has performed numerous experiments to show that the nervous system becomes infected at a very early stage.

He thus concludes that

(1) for some time before the onset of visible symptoms there exists in rabies a stage in the blood or latent stage which is not manifested by any symptom except perhaps by a few attacks of fever;

(2) at this stage the female may be quite capable of transmitting the disease via the placenta to her progeny. It appears justifiable to infer that the animal is also capable of infecting man or other animal at this stage by means of its saliva.

Confirmation of this last point by experimental methods would appear to present considerable difficulties inasmuch as it would seem to indicate the use of a "street" virus of moderate or slight virulence. Hence, the period of incubation would be of long duration and consequently also the period in which the saliva would have to be collected and injected. During this latent stage the virus certainly does not pass into the blood except in very small quantity, and thus a large number of guinea-pigs would have to be inoculated with a considerable quantity of saliva and the inoculation would have to be performed either intra-muscularly or subcutaneously; these animals would thus have to be observed for a long period, and a considerable accidental mortality would occur on account of septicaemia.

REMLINGER (P.). **L'hérédité dans l'étiologie de la rage. Explication de certains cas de "rage spontanée" des jeunes chiens.** [Heredity in the Etiology of Rabies. Explanation of Certain Cases of "Spontaneous Rabies" in Puppies.]—*Bull. Soc. Cent. Méd. Vét.* 1919. June 5. *Rec. Méd. Vét.* 1919. May 30–June 30. Vol. 95. Nos. 10 and 12. pp. 196–200.

In this short note the author submits to a further analysis the results obtained by KONRADI and himself in experiments upon the hereditary transmission of rabies, and draws from the results obtained evidence which appears to show that this hereditary transmission may be the explanation of certain cases of spontaneous rabies in young dogs. The author is of opinion that in certain conditions and in certain countries in the East where dogs are not kept under observation and the risks of infection from animal to animal are considerable heredity is by no means a negligible factor in the etiology of canine rabies. He concludes that the young dog may, even though perhaps only exceptionally, contract rabies without having been bitten, scratched, or licked by a rabid animal. The rabies thus does not manifest itself "spontaneously" but is transmitted hereditarily from the mother, which after having been infected by a rabid animal may die of rabies before its progeny or it may survive them. One may observe in such instances several dogs belonging to the same litter, or even the whole litter, contract the disease simultaneously. The multiple infection and the simultaneous onset of the disease in the young animals appears to lend further confirmation to the view that the affection is hereditary in origin.

CORNWALL (J. W.). **Anaphylactic Reactions in the Course of Anti-rabic Treatment.**—*Indian Jl. Med. Res.* 1919. Jan. Vol. 6. No. 3. pp. 237–247. With 2 figs. and 2 charts.

The author, working at the Pasteur Institute of Southern India, Coonoor, noticed for many years that patients differed considerably in the way they reacted to the antirabic injections; some, particularly healthy soldiers, coolies and children, suffered little inconvenience, whereas others, particularly the alcoholic, obese and otherwise unhealthy were considerably troubled by local pain, irritation, swelling, and perhaps subcutaneous haemorrhages. In this paper the author discusses concisely a considerable number of clinical observations and results of experimental work and appends the following summary:

"1. During the course of antirabic treatment there may occur one or more of four distinct effects which are given in the order of their frequency:—

"(1) Local cutaneous and subcutaneous tissue reaction.

"(2) General dermal reaction.

"(3) General systemic reaction in the form of malaise.

"(4) A reaction of the central nervous system in the form of temporary heart failure, occipital pain and nausea.

"2. It is suggested that these reactions are anaphylactic in nature. The antigen may be either normal brain matter or the products of metabolism of the rabies organism.

"3. Attempts to reproduce the central nervous system reaction, 1(4), in rabbits have so far been unsuccessful.

"4. Similarly, attempts to demonstrate a state of shock in sheep were without success.

"5. In untreated sheep intravenous injections of fixed-virus brain extracts caused greater disturbance of the cardiovascular and respiratory centres than extracts of normal brain substance.

"6. These disturbances are not invariable whether fixed-virus brain extracts or normal brain extracts are injected."

GRIFFITHS (J. A.). **Rinderpest Campaign in East Africa. Report of Rinderpest Operations on the Nyasa-Rhodesia Border: Sept. 1918.-Jan. 1919.**—M.S. Received from the Colonial Office 2nd June 1919. 17 pp. F'cap.

In the preceding number of this *Bulletin* (p. 92) an extract is given from the Supplementary Report dated 21st September 1918 of the Rinderpest Commission in which it is stated that rinderpest had again unfortunately broken out in the "immune belt" which, under the direction of Colonel GRAY, had been established between Lakes Nyasa and Tanganyika so as to prevent the spread of this disease southwards from what was formerly German East Africa into Rhodesia and Nyasaland and thence possibly into South Africa. Since the termination of the operations to complete the belt in July 1918 the staff was reduced to two veterinary officers, three stock inspectors, and five Europeans for guarding the belt together with African native veterinary police and border guards.

In September 1918 some movement of cattle took place from the Fort Hill section of the North Nyasa District across the Songwe River, which at that season of the year can be forded at any part of its course, into the Bundali section of the Province of New Langenburg. This movement was the result of the calling-up of the natives in the former area for military service, and in order to avoid conscription they migrated to the other side of the border and in many cases took their cattle with them. These animals were taken across stealthily by night, the natives even resorting to tying hides on the feet of their cattle in order to conceal hoof marks.

The cattle in the Fort Hill area were chiefly the animals removed from what is now the "cattle-free" belt, that is, the country between the Songwe River and the Stevenson Road—a belt of territory laying farther north. The natives were apparently tempted to slip their cattle across the border owing to the lack of watering facilities in the country in which they had been forced to move their animals.

At the end of October 1918 German troops under General von Lettow-Vorbeck crossed the above border—that is, also, the immune and cattle-free belts—near Fife into Rhodesia and were followed by British troops; about 800 head of cattle were taken across the border for feeding the troops of the opposing forces. Many of these cattle came from the Mbozi district of the New Langenburg Province, where the whole of the cattle had been immunised in May 1918, but at least half of these herds had been collected in the Wassanga country around New Utengule and in the Wabena country to the east around Njombe and to the south of this place, which areas contained rinderpest in an enzootic form. Within ten days after the enemy's cattle had been surrendered cases of rinderpest were definitely diagnosed.

Several deaths were also reported by our troops as having occurred after the cessation of hostilities among animals driven after the column for the food supply of troops.

These troops were met at Abercorn, i.e., towards the western end of the border, and as soon as the surrender took place a quarantine camp was formed just south of that place. Since rinderpest was found in this camp all the susceptible in-contacts were destroyed and the cattle in the country for 14 miles around were injected with anti-rinderpest serum. As soon as the armistice was concluded a search was made along the routes that had been taken by the troops for stray cattle or signs of disease in the local stock. Search was also made along the Abercorn-Kasami Road, and an account is given of searches made in other directions.

In November rinderpest was found to have broken out among susceptible animals at Isoko (that is, north of the Songwe River and nearly due south from New Langenburg). These animals were some of a number brought over from Nyasaland in June 1918 to test a reported outbreak of the disease among immunised animals. The disease had apparently been introduced into Isoko by an animal brought in for sale from a village in the Bundali section—an animal that had not been submitted for inoculation at the time these operations were being carried out in the district. The sick animals were isolated and fresh susceptible animals obtained from Nyasaland in order to diagnose the disease with certainty. The disease was definitely ascertained to be rinderpest and the opportunity was then taken to test the immunity of animals that had been immunised in the ordinary course of operations.

These tests were carried out (1) by placing some immunised animals in the same cattle sheds as the sick and (2) by inoculating another lot with virulent blood and keeping them isolated apart from the sick, with separate attendants and grazing ground. In no case did any animal that had been previously immunised show any reaction. A number of charts showing the temperatures of these animals after inoculation and also of the susceptible controls inoculated to test the virulence of the virus used are appended.

Two susceptible animals inoculated with virulent blood were simultaneously infected with anti-rinderpest serum; these showed subsequently only thermal reactions. The dose of serum was at the rate of 10 c.c. per 100 lbs. body weight in one case and 7½ c.c. per 100 lbs. in the other. An experiment of the same kind was carried out with two animals of approximately the same weight, which were inoculated with serum in the same doses as in the case of the above two but were not given any virulent blood. These were placed in contact with infected animals and subsequently showed only a thermal reaction.

It is reported that a thorough survey was being made of the cattle in the Karonga area, that is on the western shore of Lake Nyasa, this being the most densely populated part of the country south of the Nyasa-Rhodesia border. The human influenza epidemic was causing a good deal of inconvenience to the workers of the Commission.

At the time of writing (9th January 1919) Griffiths states that all reports received indicate that all cattle south of the "cattle-free" belt were apparently free from the disease so that the situation may

be considered to be satisfactory. Attention, is, however, called to the fact that rinderpest is still active to the north of the immunised area and also that infected herds have traversed the portions of Northern Rhodesia between Fife and a point 50 miles south of Kasama and thence up to Abercorn. Also, although a number of stray cattle lost by the troops on the routes they followed have been captured by patrols and destroyed, there is the possibility that the natives who might have found stray animals would conceal this fact and in this manner the disease might be kept alive in some remote part of Rhodesia at some distance away from the routes taken by the troops in their passage through the country. A supply of serum is being kept on hand to deal with possible emergencies.

In an appended letter, dated 15th March 1919, to the Imperial Secretary, High Commissioners Office, Capetown, from the Rinderpest Commissioner (Colonel C. E. GRAY) it is stated that instructions had been sent to the officer in charge of the Commission to close everything down at the end of the month if the situation continued to be satisfactory.

SCHERN (Kurt), MAVRIDES (Nicolaki) & MAJOR (Alexander). **Ueber Rinderpest. (Dritte Mitteilung.) Ueber Resistenz und Inkubation bei der experimentellen Rinderpest anatolischer und podolischer Rinder.** [Rinderpest Third Report. The Resistance to Infection and Period of Incubation in Experimental Rinderpest of Anatolian and Podolian Cattle.]—*Ztschr. f. Infektionskr. . . . d. Haustiere.* 1919. June. Vol. 20. No. 2. pp. 117–121.

In this short paper the authors summarise their observations in the above connection upon a considerable number of cattle employed by them in anti-rinderpest serum production at the Bacteriological Institute, Pendik, Asia Minor. They conclude that the period of incubation of the disease as it affects Anatolian and Podolian cattle in the East may often last longer than the usually stated interval of 3 to 9 days, at least it very often extends to 10 days and may even reach 15 days.

Over 20 per cent. of both these breeds of cattle in Asia Minor proved to be immune against heavy artificial infection with rinderpest.

The findings of the International Veterinary Congresses held in the years 1863, 1865, and 1876, and also of the International Conference of Hygiene held in 1872, according to which the maximum duration of the incubation period in rinderpest was laid down to be 9 days, thus can no longer be held to be correct in view of the observations of ANGELOFF and the authors themselves. According to the authors, out of 1,826 animals suffering from rinderpest, symptoms of the disease were shown in 89 head (=4·87 per cent.) later than 9 days after infection.

From a practical point of view these observations are of importance inasmuch as they indicate that in the case of Anatolian or the Podolian breeds of cattle a quarantine period of 10 days cannot be deemed to be sufficiently long for the prevention of the spread of rinderpest when these animals are imported into rinderpest-free countries.

CURASSON. **Note sur la production rapide de sérum contre la peste bovine et son utilisation en région infectée.** [The Rapid Production of Anti-Rinderpest Serum and its Utilisation in an Infected Region.]—*Rec. Méd. Vét.* 1919. June 15. Vol. 95. No. 11. pp. 323–327.

In this article the author gives an account of a rough and ready method employed by him in the manufacture of anti-rinderpest serum in French West Africa.

The animals employed for hyper-immunisation are preferably those which have recently recovered from a natural attack. The author is very doubtful about the value of vaccination with bile inasmuch as the virus contained in it is probably not at all attenuated. The animals chosen should be adults—young animals bleed badly—and in as good condition and of as large size as possible. One is able to identify definitely recovered animals by the presence of retrogressive lesions such as rinderpest eczema, ocular lesions, necrotic lesions of the skin and ears, chronic vaginitis, etc. Milch cows, vicious animals and those affected with trypanosomiasis should be excluded. The serum-producers thus hired from their owners are placed in an enclosure and the problem of feeding them becomes somewhat difficult especially during the dry season. The ordinary jungle straw which is innutritious should not be fed if possible but one should substitute for it more nourishing foods such as the fruits of " n'gouin " (*Pterocarpus erinaceus*) while still green, soft, containing very nutritious seeds and much liked by the animals, or the fruits of " sindia " (*Cassia sieberiana*) which are also nutritious and drier but are useful for resting animals inasmuch as they possess slightly laxative properties. The fruits of " gouro " (*Acacia sp.*) are also very appetising. Bulky food may be supplied in the form of bamboo leaves, or the young sprouts of andropogon gathered around marshes according to the district. A plentiful supply of drinking water should be provided.

Virulent blood is obtained from animals during different stages of the disease; the choice of these animals depends only upon their condition. Each lot of virulent blood is taken from several animals at the same state of infection and the mixture of these various bloods is used for inoculation immediately. It is preferable not to bleed the animals to death for a number of affected animals are not always at hand and it may be necessary to bleed the same animal several times. Moreover, a bad impression would be produced upon the native by sacrificing any of his beasts even if compensation were granted, and besides the chances of recovery although remote can not be eliminated. The virus-producers are paid for in the same way as infected or in-contact animals destined for slaughter.

In the hyper-immunisation of his serum producers the author gives two inoculations of rinderpest blood, the first taken from an infected animal towards the end of the first period of the disease and the second from an animal in the second or third period, the avowed object being to obtain a serum which is both anti-toxic and anti-microbial. Each inoculation, which consists of three or four litres of blood, is sufficient in itself to produce hyper-immunity and this fact gets rid of the objection that the second injection is perhaps not virulent. The rinderpest blood is withdrawn from an animal

that has been cast, and run into sterile flasks containing two grammes of sodium citrate in 5 per cent. dilution. If an autoclave is not available for sterilisation then this can be done by boiling in a pan containing 2 per cent. sodium borate. The solution boils at about 105° C. and disinfection can be accomplished with certainty almost by two successive heatings at one hour's interval. From three to five litres of blood are taken from each animal depending upon its size. The mixture of bloods is inoculated immediately, the animal to be hyper-immunised being cast and five areas about as large as one's hand disinfected at the base of the neck, behind the shoulder, or in the flank. The blood is allowed to run in subcutaneously at these sites by the force of gravity through a rubber connection from a sterile funnel suspended at a height. The wounds left after withdrawal of the canula are closed by means of a pin and thread. If care is taken with regard to sterility of the implements no untoward results occur subsequently. The second inoculation is made four days afterwards in the same way into the other side of the body.

Blood is first withdrawn from the hyper-immunised animals ten days after the second inoculation and three more bleedings are performed at four day intervals, from three to five litres of blood being taken on each occasion according to the size and condition of the animal. The blood is taken from an animal in the recumbent position and run into sterile bottles containing 2 grammes potassium oxalate and 25 centigrammes of carbolic acid dissolved in 50–60 c.c. of water. The cotton wool plugs are then removed from the bottles and are replaced by cork stoppers, sterilised and then paraffined. Operating beds of wicker work or bamboo can be made for casting the animals to avoid contamination with dust and these beds should be sprinkled with water after each bleeding. Inoculations with virulent blood may be recommenced after 8–10 days' rest.

If one desires to obtain serum instead of oxalated blood the following method may be employed. Ordinary bottles as large as possible are taken and one places in them some glass tubes, the walls of which have been perforated with holes from 7–8 mm. in diameter by means of a painter's lamp, and of about three-quarters the length of the bottle. Three tubes of small diameter are passed through the stopper; one of these serves for the entry of blood and is tied with thread or closed with a clamp after admitting the blood, which should enter below the upper end of the perforated tubes. The second provided with a small length of rubber tubing serves for running off the serum, whilst air penetrates into the bottle, filtered through a cotton wool plug by the third tube. The vessel so prepared is sterilised and it is stated that from 36–48 hours after it has been filled serum can be obtained in the proportion of 60 to 65 per cent. of the total volume of the fluid. This serum is distributed in bottles containing carbolic acid dissolved in a little water in the proportion of 25 centigrammes per litre of serum.

Although the result of experiments made in India indicate that anti-rinderpest serum is affected very little by heat it appears best to keep the blood or serum in a cool medium. A cubical trench at least one metre each side is thus dug in a shady place and the walls covered with a layer of straw about 15 cm. in thickness enclosed between two pieces of basket work. A thick lid of straw shuts up the

hole; the floor also is covered with a layer of straw, the whole is covered with a straw roofing. By frequently watering the straw walls of this receptacle one obtains a permanent lowering of temperature. For transportation the blood or serum bottles are placed in baskets of bamboo or palm twigs filled with damped straw which surrounds and covers the bottles.

Virulent blood, used for vaccinations, is also kept in a cool place. Attempts were made to preserve it by passing through sheep ; infection succeeded in one case out of four. As regards the goat the results are still more uncertain, contrary to the statements of SCHEIN [see this *Bulletin*, Vol. 6, No. 1, p. 32]. The goats and sheep of French West Africa are probably particularly resistent.

Use of blood or of Serum for Immunisation.

Preventatively, the use of serum alone or of the serum-virus method depends upon circumstances. In the scene of a scattered outbreak of a spreading nature one must have recourse to a large extent to the serum-virus method. But if one has to deal with the beginning or with the end of an outbreak in a well circumscribed area or with little tendency to spread it appears advisable to operate in the following manner. The herds immediately surrounding the focus of rinderpest are immunised by means of serum and virus. In a zone further away from the centre one employs serum alone a few days later, that is to say, a little prior to the time when slight manifestations of rinderpest should appear in the animals of the first zone. The dose of oxalated blood, inoculated into an adult animal in the recumbent position, is 40 c.c., for young animals 20–30 c.c. Instead of a syringe one may employ an ordinary bottle fitted up like a wash bottle for the inoculation. The virus inoculated simultaneously in doses of $\frac{1}{4}$ c.c. per head consists of blood from an affected animal in the febrile stage and kept liquid by defibrination or the addition of 0·2 per cent. sodium citrate. This is kept in a cool place and can only be utilised for a few days after it has been taken. The author recommends the inoculation of the oxalated blood underneath the soft skin situated just behind the elbow and the injection of virulent blood just in front of the shoulder. If instead of oxalated blood one employs serum the doses can be diminished by half. Curatively the serum is limited in its application. It is only certainly effective during the first period of the disease. During the second and third period, or in animals suffering from the sub-acute form one may obtain positive results after the employment of large doses (200–500 c.c.) in about half the cases.

DORSET (M.), MCBRYDE (C. N.), NILES (W. B.) & RIETZ (J. H.). **Studies on the Hyperimmunisation of Hogs against Hog Cholera.**— *Jl. American Vet. Med. Assoc.* 1919. June. Vol. 55. (New Series. Vol. 8.) No. 3. pp. 259–280. With 10 tables.

The serum now employed on a large scale in the prevention and treatment of swine fever is produced from pigs that have been rendered immune and then subsequently injected with large quantities of defibrinated blood obtained from pigs at the height of an attack of swine fever. The blood of the hyperimmunised animals is then drawn, a preservative added, and this fluid constitutes the serum

used in practice. In 1908 the first three of the above authors published a report upon the methods that might be adopted for hyperimmunising the pigs. This process, it was stated, might be carried out in two ways, viz., (1) by the quick method wherein the immune pig was injected subcutaneously with an amount of virulent blood equivalent to 10 c.c. per lb. body weight, and (2) the slow method in which the immune pig was first injected with 1 c.c. of virulent blood subcutaneously per lb. body weight, followed after an interval of 10–14 days by a second dose amounting to 2½ c.c. per lb. body weight and again after about 12 days a third injection in the proportion of 5 c.c. per lb. body weight. Both these methods, however, present certain objections. The quick method, involving the subcutaneous injection of very large amounts of defibrinated blood, is difficult to perform and frequently results in the development of large subcutaneous abscesses.

In the slow method abscesses were likewise sometimes produced and in addition a very long period—32 days at least—elapses before the pig could be bled. These objections, however, were entirely eliminated by resorting to the intravenous method of administration and it was shown (1911) that immune pigs could be hyperimmunised satisfactorily by the administration of a single dose of virulent blood intravenously in the proportion of 5 c.c. per lb. body weight. In earlier experimental work it was shown that a potent serum could be obtained by the hyperimmunisation of immune pigs whether the immunity was set up artificially by the injection of virus and serum, or by recovery from an attack of the disease, or was a natural immunity. In this early work the influence on the potency of the serum of the interval of time elapsing between immunisation and hyperimmunisation was not studied although it was found that a satisfactory serum could be produced when the interval was as long as 13 months. In this connection the authors then discuss certain results published by Holmes (1910–1911) bearing upon alleged improved methods in the preparation of anti-rinderpest serum. Prior to the publication of Holmes's experiments this serum was usually prepared by inoculating susceptible cattle simultaneously with virulent blood and a suitable dose of anti-rinderpest serum; about three weeks later the animals were given one large injection of defibrinated virulent blood in the proportion of 10 c.c. per lb. body weight, or were given a first injection of about 3·5 c.c. per 1 lb. body weight, followed after two or three weeks by a second injection in the proportion of from 7–10 c.c. per lb. body weight, all these injections being made subcutaneously. As the result of his experiments Holmes maintained that an animal that had been immunised against rinderpest by the simultaneous inoculation of serum and virus passed through a period of hyper-susceptibility called the " negative phase " ; this was followed by a period of resistance to the disease, the " positive phase." Accordingly, if the process of hyperimmunisation was carried out three weeks after the simultaneous inoculation the cattle received the massive quantity of virulent blood after they had passed through the negative phase and were in the positive phase of the immunity. Holmes's view was that these large doses should be injected during the negative phase of immunity, that is, before the body cells of the animal had produced the antitoxin to a considerable degree; the

body cells would thus be stimulated to the maximum degree and a serum of maximum potency would be obtained. If, on the other hand, the hyperimmunising injection was made during the period of positive immunity the virus injected combined in part with the antitoxin that had been developed as the result of the previous immunising dose and thus the cells concerned in antitoxin production would not be stimulated to the desired extent.

DORSET and his collaborators describe experiments in which the applicability of HOLMES's hypothesis was tested in respect of the production of anti-swine fever serum. In these experiments a number of susceptible pigs were first given simultaneous inoculations of serum and virus and were later hyperimmunised after intervals of 1, 2, 5, 7, 9, and 10 days, and 3, 4, 5, 6, 7, and 8 weeks, respectively. The pigs hyperimmunised after the 1, 2, and 5 day intervals each received 5 c.c. of virulent blood per lb. body weight. In the case of the 7, 9 and 10 day intervals one pig in each case was given the amount ordinarily employed for hyperimmunising, viz., 5 c.c. per lb. body weight while another pig was given only half that amount, i.e., only 2·5 c.c. per lb. body weight. In the case of the longer intervals one pig in each case was given 5 c.c. of virus per lb. body weight. These pigs, termed "short interval immunes" weighed from 95 to 205 lbs. at the time of hyperimmunisation.

The samples of virulent blood employed in these experiments were also used for the hyperimmunisation of what the authors call "regular immunes;" these were pigs that had been employed in determining the potency of various samples of anti-swine fever serum when they weighed only from 40-65 lbs. and having shown no symptoms of illness following this treatment with serum and virus were kept from 3 to 6 months, when they had attained from 163–220 lbs. in weight, before being submitted to the process of hyperimmunisation. These "regular immunes" which were hyperimmunised in order to secure a control on the antigenic properties of each of the lots of virus used in the hyperimmunising of the "short immunes" were administered the hyperimmunising doses and blood on the same days as the corresponding "short interval immunes" and the sera thus obtained were tested in the same way. The pigs were bled in all cases two weeks after hyperimmunisation and serum was prepared in each case in the usual manner by defibrinating the blood and adding 0·5 per cent. of phenol.

The above sera were tested on susceptible pigs weighing from 35 to 85 lbs. each. Six pigs were used in testing each serum, two receiving 5 c.c., two 10 c.c., and two 15 c.c. each, subcutaneously. Each pig at the same time received a subcutaneous injection of 2 c.c. of virulent blood in the groin opposite to that in which the serum was injected. A certain number of the pigs were injected with the virus alone to serve as controls.

The results, which are depicted in tabular form, are very striking. Every one of the 11 regular long-interval immune pigs yielded a serum of high potency while only one pig out of the 13 that were immunised within six weeks after simultaneous inoculation yielded a serum potent enough for use in practice. There was an almost complete lack of potency in the sera obtained from pigs that were administered a hyperimmunising dose within ten days after they had been immunised; of the 9 pigs hyperimmunised during that period 3 were

given only half as much virus per lb. body weight as was given to the other pigs in this group and to the regular long-interval immunes. These three pigs are therefore excluded from consideration in the discussion of the results. Out of 36 susceptible pigs inoculated with the sera from the six pigs that had received 5 c.c. of virulent blood per lb. body weight 29 or 80·5 per cent. died of swine fever. Some of these sera showed evidence of a slight protective value but in none of them was this manifested in a degree comparable to that reached by the sera from the regular (long-interval) immunes.

The sera from the pigs that were hyperimmunised from 3 to 6 weeks after immunisation were somewhat more potent than those from the preceding group of pigs, but they were, on the whole, distinctly inferior to the sera obtained from the regular immunes. Of 24 pigs used to test these sera five, or 20·8 per cent., died of swine fever in spite of the fact that some of them had received the larger doses of serum (10–15 c.c.).

The sera from the pigs that were hyper-immunised seven or eight weeks respectively after immunisation appeared to be of satisfactory potency inasmuch as only one test pig out of 12, 8·3 per cent., died and that one received the smallest dose of serum.

The sera obtained from the 11 regular (long-interval) immunes, and tested comparatively in groups along with the sera from the short interval immunes, gave the following results :—Out of a total of 66 test pigs two (3·3 per cent.) died during the tests ; these two each received only the smallest dose of serum.

In the case of the control pigs inoculated with virulent blood alone during these tests symptoms of swine fever were set up in all cases, a number were killed, before death would have actually taken place in order to obtain virulent blood, but it is estimated from past experience that at least 95 per cent. would have naturally succumbed.

From these results it is evident, the authors state, that in the case of swine fever hyper-immunisation following shortly after immunisation, or during the so-called negative phase, is not to be recommended inasmuch as it results in the production of very inferior serum. It appears that, as a rule, and within certain limits, the longer the interval that elapses between immunising on the one hand and hyper-immunisation on the other, the more potent the serum. These results were surprising in view of HOLMES'S results in producing a highly potent anti-rinderpest serum by hyper-immunising at the negative phase within 10 days after immunisation. The authors find a considerable analogy between their results and the findings of PARK and ZINGHER in their work on the active immunisation of children against diphtheria by the injection of mixtures of toxin and antitoxin.

In his publications upon improved methods for the production of anti-rinderpest serum HOLMES further reported that virulent rinderpest blood diluted with hypotonic citrate solution was more satisfactory for hyper-immunisation than the undiluted defibrinated blood. He believed this was due in part to the elaboration of the virus from the red blood corpuscles through haemolysis and in part to the more rapid absorption of the diluted virus, the hyper-immunising doses in the anti-rinderpest serum production being given simultaneously. In the production of serum against swine fever the hyper-immunising dose is given intravenously; hence rapid dissemination even of the

undiluted defibrinated blood is assured, but since there was a possibility that through haemolysis a greater amount of the antigen would be made immediately available the authors hyper-immunised several immune pigs with diluted virus in order to compare serum produced in that way with that secured by the common practice of injecting the undiluted defibrinated blood.

First, the authors endeavoured to ascertain the advantages to be derived from the use of blood diluted with citrate solution. Blood from pigs suffering from swine fever was mixed directly with a 0·5 per cent. solution of potassium citrate in equal amounts. A portion of the blood from these pigs was run into separate vessels and defibrinated. These two lots of virulent blood were used to hyper-immunise five regular long-interval immunes intravenously. In two cases the blood was allowed to stand for 24 hours and 3 days, respectively, in a cool place to determine whether standing or more complete haemolysis would increase its antigenic properties. The pigs were bled 14 days after hyper-immunising and each lot tested in the manner described above on susceptible pigs. Three of the immune pigs that were given the citrated virulent blood were given 5 c.c. per lb. body weight, and one received 10 c.c. per lb. body weight, i.e., 2·5 and 5 c.c. of actual virulent blood; one immune pig was hyper-immunised with 5 c.c. per lb. body weight of undiluted virulent blood. Of the 24 pigs used to test the potency of the sera from the immune pigs that had received the citrated blood 18, or 75 per cent. became sick and 4, or 16⅔ per cent. died. Of the six pigs that were given serum from immune pigs hyper-immunised with undiluted defibrinated blood all (100 per cent.) remained well. Four control pigs injected with virulent blood alone developed acute swine fever in all cases.

The tests showed that the serum obtained from the immune pig that had been given the largest dose of citrated blood was no better than that obtained from those that had received half as much of the same blood. The serum from the immune pig immunised with undiluted virulent blood was distinctly more potent than those obtained from the pigs immunised with citrated blood.

Secondly, the comparative value of virulent blood diluted with water was tested. If the antigenic properties of virulent blood could be increased by the dissolution of the red blood corpuscles it would be reasonable to expect that the degree of increase would depend upon the extent of the dissolution. The authors endeavoured to obtain a more complete haemolysis by drawing virulent blood direct into distilled water, and by mixing defibrinated virulent blood with distilled water. The methods are described by means of which laked blood was prepared from four swine fever affected pigs in both these ways, and then the potency of the serum obtained after hyper-immunisation of a pig in each case with this material was tested upon susceptible pigs and compared with that obtained following upon immunisation with undiluted defibrinated blood. No apparent advantages were demonstrable by the use of laked blood in hyper-immunisation.

[Although the authors in this paper discuss at length the methods of hyper-immunisation recommended by HOLMES in the production of an anti-rinderpest serum and institute comparisons with the results

obtained by themselves in the case of swine fever it is not by any means improbable that the processes do not represent analogous phenomena.—ED.]

NEGRETE (J.) & KANTOR (L.). **Parálisis bulbar infecciosa Aujeszky (pseudo-rabia, peste da Coçar).** [Infectious bulbar paralysis (Aujeszky).]—*Rev. Inst. Bacteriologico*, Buenos Aires. 1918. Jan. Vol. 1. No. 2. pp. 205–218.

Infectious bulbar paralysis was first observed and investigated by AUJESZKY in cattle in Hungary. In South America it has so far been found only in Brazil in the provinces of Minas, Matto Grosso, Rio de Janeiro and San Paulo, where it has been studied by CARINI and MACIEL (*Centralblatt f. Bakteriol*: Bd. 32). The disease is known in Brazil under the names *pseudo-rabia* and *peste da Cocar*—the former because dogs affected sometimes pass through an aggressive stage. It has, however, no affinity with rabies, its symptomatology being entirely different. BAUZA and NEGROTTO describe an epizootic of rabies in horses and cattle in the province of Santa Catalina in Brazil (*Revista de Medicina y Veterinaria de la Escuela de Montevideo*). Some of the accounts given by agriculturists are suggestive of infectious bulbar paralysis. The disease has not so far been observed in the Argentine. It was at first attributed to poisoning by certain toxic herbs. It has, however, been clearly proved to be an infectious disease produced by a filterable virus, the present authors having produced it in animals by inoculating them with infected urine passed through a Berkefeld filter. In the course of a laboratory study of the disease the authors found the rabbit the most easily infected animal. The goat and the domestic fowl are refractory and, according to AUJESZKY, the pigeon also. The authors, however, state that they have succeeded in communicating the disease to the pigeon by intra-cerebral injection. The medulla is the only organ whose virulence is constant. The virus has a special affinity for the central nervous system and intra-cerebral inoculation will sometimes succeed when every other method has failed. Rabbits have become infected when placed with infected animals. The nasal secretion and the urine are infectious. Attempts at immunization have not been successful.

MISCELLANEOUS.

VALLERY-RADOT (Pasteur) & LHÉRITIER (A.). **Etude sur la pathogénie de la fièvre bilieuse hémoglobinurique des bovins en Algérie.** [The Pathogenesis of Bovine Biliary Haemoglobinuric Fever in Algeria.]—*C. R. Soc. Biol.* 1919. Apr. 12. Vol. 82. No. 11. pp. 389–391.

A reference to this condition, which sometimes causes great losses among herds of cattle in Algeria, has already been made in the preceding number of this *Bulletin* (p. 101).

It is characterised, in its most common form, by fever, haemoglobinuria, and jaundice. Sometimes haemoglobinuria is the only

marked symptom. In other cases jaundice appears to be the only symptom of importance. *Piroplasma bigeminum* cannot be incriminated as the causal organism except in rare cases. In the great majority examination of the blood gave negative results or disclosed only small ring-shaped or rod-shaped piroplasms, which can also be seen to the same extent in healthy cattle. Experimental inoculation with the blood of sick animals gave negative results. Thus, according to SERGENT (Ed. and Et.) and Lhéritier (A.), the etiology of this form of bovine redwater remains unknown.

In this note the authors discuss only the pathogenesis of the disease. Observations made at different times during the course of the disease indicate that it passes through a stage of haemoglobinaemia with jaundice and then a stage of jaundice. Blood examinations showed that at the commencement of the disease there occurs a haemolytic process; in the stage of haemoglobinaemia the corpuscles exhibit a very marked fragility; in two cases described haemolysis commenced when the corpuscles were suspended in a sodium chloride solution of 0·86 per cent. saturation, whereas in normal cattle it only commences between 0·58 and 0·68.

In this connection it is interesting to recall the results obtained by NATTAN-LARRIER and PARVU in their studies on canine piroplasmosis. These authors discovered that the red corpuscles showed a markedly diminished resistance when blood-tinged urine commenced to be passed.

The haemoglobinaemia is accompanied by a considerable degree of anaemia; the blood at this stage shows hardly any signs of regeneration and it sometimes exhibits red corpuscles with basophile granules.

The jaundice is of a special type comparable to that seen in the types of haemolytic jaundice described in human pathology. The urine does not contain or contains only traces of bile pigments; bile salts are absent. The faeces are not discoloured and on post-mortem examination a marked increase in size of the spleen is observed but no granular red corpuscles are seen and these corpuscles do not show any autoagglutination. No noteworthy lesions are seen in the liver; no iron pigment is discoverable in this organ.

The authors then give a detailed account of two observations and a further discussion of the significance of the results obtained.

VAN SACEGHEM (R.). **Contribution à l'étude de l'Ostéoporose au Congo belge.** [Osteoporosis in the Belgian Congo.]—*Bull. Soc. Path. Exot.* 1919. May. Vol. 12. No. 5. pp. 238–243.

In this paper the author first discusses the theories advanced to account for the above condition in horses and then recounts a few facts concerning the disease obtained by him in the course of investigations which he had commenced to undertake in the Congo but which he could not unfortunately carry out. The disease appears to be very widespread in the Belgian Congo, and has been observed at Congo da Lemba, Kitobola, Miao, Nyangwe, La Tjopo, Stanleyville, Panbili, Ye Pordo, La Gada, and Dungu. All species of equidae are susceptible to the disease but horses seem to become more rapidly affected than donkeys. Equines kept permanently in stables or living at liberty in the jungle become affected in the same proportions.

The source of the equidae introduced has no effect upon the course of the disease. A healthy horse introduced into a district where osteoporosis was enzootic became infected after five months. The disease varies considerably in duration and may last from a few months to over a year. Fever is not observed but the animals before showing the characteristic symptoms of osteoporosis manifest lameness and paresis. On post-mortem examination the principal lesions are found in the bones; the medullary canal is enlarged whilst the compact bone diminishes in thickness. The bone becomes flexible, lighter than normal, easily broken, and becomes so soft that in some cases it can be cut with a knife; the bone marrow becomes red and dotted over with ecchymoses. The bones of the head are especially affected.

The affected animals harboured intestinal worms, but these could be of no significance inasmuch as all horses in the Congo harbour numerous parasites (oxyurids, Habronema, Cylicostomum, Gastrodiscus, *Strongylus equinus*, and other worms). All the horses affected with osteoporosis were well fed; they received maize, rice, guinea grass, false sugar cane, soya bean, etc. Well marked lesions of osteoporosis were seen even in colts 8–9 months old.

It is stated that in all the districts in which the disease occurred analysis of the soil showed that the earth from these parts contained very little calcium and phosphorus. Soluble calcium was present only in very small amount or was altogether absent in the waters of these districts (Congo da Lemba, Kitobola, Nyangwe). On the other hand, at Zambi where a considerable number of horses were bred and no cases of osteoporosis had been observed the waters were found to contain a considerable quantity of calcium in solution.

These results would indicate that calcium deficiency is the cause of osteoporosis in the Belgian Congo. However, the cattle and sheep of these regions showed no symptom or lesion of osseous cachexia. It may be that the equidae are more susceptible to calcium deficiency than other animals.

The author holds that definite proof was obtained with regard to the role played by calcium salts in the drinking water and not by the calcium fixed in various forms in foods, by the following fact: osteoporosis was completely eradicated from a district in which it prevailed as an enzootic by giving horses water to drink containing calcium acid carbonate in solution.

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